Chapter 7

The Impact of Plant-Parasitic Nematodes on Agriculture and Methods of Control

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Additional information is available at the end of the chapter

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Abstract

Plant-parasitic nematodes are costly burdens of crop production. Ubiquitous in nature, phytoparasitic nematodes are associated with nearly every important agricultural crop and represent a significant constraint on global food security. Root-knot nematodes (*Meloidogyne* spp.) cyst nematodes (*Heterodera* and *Globodera* spp.) and lesion nematodes (*Pratylenchus* spp.) rank at the top of list of the most economically and scientifically important species due to their intricate relationship with the host plants, wide host range, and the level of damage ensued by infection. Limitations on the use of chemical pesticides have brought increasing interest in studies on alternative methods of nematode control. Among these strategies of nonchemical nematode management is the identification and implementation of host resistance. In addition, nematode genes involved in parasitism represent key targets for the development of control through gene silencing methods such as RNA interference. Recently, transcriptome profiling analyses has been used to distinguish nematode resistant and susceptible genotypes and identify the specific molecular components and pathways triggered during the plant immune response to nematode invasion. This summary highlights the importance of plant-parasitic nematodes in agriculture and the molecular events involved in plant-nematode interactions.

Keywords: plant-parasitic nematodes, agriculture, crops, genetics, resistance, *Meloidogyne*

1. Introduction

Over millions of years, the association of plants and nematodes has resulted in the evolution of the plant-parasitic nematode. Widely distributed pathogens of vascular plants, enormous losses in yields have been attributed to the presence of nematodes. The intricate relationship

between the parasitic nematode and plant has culminated in an "evolutionary arms race". Phyto-parasitic nematodes have evolved strategies to suppress host immune responses for the development of feeding sites. In turn, plants have developed specific molecules to recognize pathogens signaling the activation of immune responses. Declining use of chemical pesticides has brought great attention to research in alternative methods of nematode control. An effective strategy for nematode management involves the utilization and implementation of nematode-resistant cultivars into crop breeding programs. Currently, genetic sequencing analyses are widely utilized in the identification of molecular components of nematode parasitism and is also used to distinguish nematode-resistant and susceptible plant genotypes. These detailed analyses have significantly contributed to our overall understanding of the dynamic and complex nature of plant-nematode interactions.

2. Nematode morphology

Nematodes are a fascinating, biologically diverse group of organisms. Their ability to adapt to a wide variety of habitats including; marine, soil and aquatic, provides an evolutionary advantage for species longevity. Phylum Nematoda is largely distinguished by three major monophyletic groups including: Enoplia (marine), Dorylaimia (parasitic trichinellids and mermithids and Chromadoria (nematodes of various environments). Nematodes belong to the group Ecdysozoa, which comprises animals that can shed their cuticle. Over 30,000 species of round worms are found in Nematoda [1] typically ranging in size from 0.2 mm to over 6 m. Nematode body structure is relatively simple and characterized as limbless, cylindrical, and elongated. Essentially the body plan is a "tube within a tube", the inner tube or alimentary canal, consists of a digestive tract and gonad which are surrounded by an outer tube; a body wall containing a series of dorsal and ventral longitudinal muscles attached to the hypodermis. These muscles are activated by the dorsal and ventral nerves and their contractions allow for locomotion in sinusoidal waves. In plant-parasitic nematodes, a primary infection structure called a stylet is located at the anterior end of the nematode which is followed by an esophageal region that connects the stylet to the intestines. A typical tylenchoid esophagus consists of an anterior procorpus, a median bulb and the posterior basal bulb. The median bulb functions in the transfer of enzymes involved in primary infection and facilitates the movement of plant nutrients into the intestine. Inside of the exterior body wall lies the pseudocoelom, a unlined, pressurized, fluid-filled cavity formed directly from the blastula surrounding the gut cavity. The pseudocoelom is filled with fluid which provides turgor pressure for the entire body containing the internal organs and aides in the transfer of nutrients, oxygen and metabolic products. The excretory system is composed of four distinctive cells, an excretory pore cell, a duct cell, one canal cell, and a fused pair of gland cells. Nematodes are enclosed within an exoskeleton called a cuticle which is secreted by inner hypodermal cells, and is primarily composed of collagens, insoluble proteins (cuticlins), glycoproteins and lipids. The cuticle plays an important role in movement, environmental protection and growth and development [2]. The typical male reproductive structures include a testis, a seminal vesicle and a vas deferens leading to a cloaca, while the female reproductive system is tubular containing one or two ovaries, seminal receptacles, an uterus, ovijector and a vulva.

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3. Evolution of plant-parasitic nematodes

Why do some nematodes become plant parasites? The dynamic association of nematode and plant host has resulted in plant parasitism which has evolved three times culminating in substantial benefits for nematode survival and development [3, 4]. An existing evolutionary hypothesis places the origins of these ancient microscopic roundworms around 400 million years before the explosion of animal phyla (pre-"Cambrian explosion") [5]. Evidence suggests the initial presence of plant-parasitic nematodes to have occurred around 235 BC [6] while the first described plant parasitic nematodes were reported by Needham who observed symptoms of galling in wheat [7]. An agriculturally important species of plant-parasitic nematodes called root-knot nematodes, were initially identified by Berkeley who observed the presence of galls on cucumber roots [8].

The plant-nematode association has resulted in the development of specific feeding structures and secretory products that are involved in host infection and nutrient absorption. Plant parasitic nematodes are specialized by the stylet and subventral and dorsal esophageal glands which are considered the most significant evolutionary adaptations for plant parasitism [4, 9]. Plant-parasitic nematodes utilize a hollow, needle-like, protrusible stylet to probe plant tissue and release an assortment of proteinaceous secretions from the subventral and dorsal glands which comprises the integrity of the host cell and allow for nematode entry. These glandular secretions induce cellular remodifications that are essential for development of a metabolically active feeding cell [10]. Among the secretory molecules are a group of carbohydrate-active enzymes. Since cellulose is the primary component of plant cell walls, cellulases (β-1,4-endoglucanases) are secreted to degrade the cell wall which allows nematode entry into host tissue. Genomic analyses of root-knot nematodes have revealed the presence of a suite of enzymes called CAZymes (cellulases, xylanases and other glycosyl hydrolase family members (GHFs)) [11]. Beta-1,4-endoglucanase genes have been isolated from plant-parasitic cyst nematodes with catalytic domains belonging to family 5 of the glycosyl hydrolases [12, 13].

Glycosyl hydrolase families G5 and G45 have been identified in plant-parasitic nematodes. Plant-parasitic nematode GH5 cellulases show closes homologies with bacterial G5s, which suggests an initial horizontal gene transfer of bacterial G5 cellulases into nematode genomes during the evolution of the plant-parasitic order Rhabditida (suborder Tylenchina) [14, 15]. G45 cellulases have been found in plant-parasitic nematode *Bursaphelenchus xylophilus* of the Aphelenchida order [16]. Phylogenetic analyses have shown similarities in gene structure between G45 sequences found in these nematodes and ascomycetous fungi which supports the hypothesis of a horizontal gene transfer event from fungi to nematodes [17].

Plant-parasitic nematodes differ in lifestyles. Some nematodes will invade the plant cells while others simply obtain nutrition externally. Ectoparasitic nematodes remain outside the host cells and feed on plant roots while endoparasitic nematodes establish residence within plant tissue. An example of ectoparasitic nematode is Xiphinema (California dagger nematode) which transmits the Grapevine fanleaf virus. The resulting viral infection causes tremendous economic losses in grapes worldwide [18]. Endoparasitic nematodes are further divided into migratory and sedentary groups. Migratory endoparasitic nematodes move within the root

and remove cytoplasm killing the host cell while sedentary nematodes become immobile after the development of a feeding site within the host tissue [19]. Migratory endoparasitic nematodes of economic significance include *Pratylenchus* spp. (lesion nematode), *Radopholus* spp. (burrowing nematodes) and *Hirschmanniella* (rice root nematode).

4. The impact of plant-parasitic nematodes on crops

Plant-parasitic nematodes are a costly burden in agricultural crop production. Over 4100 species of plant-parasitic nematodes have been identified [20]. Collectively, they cause an estimated $80–$118 billion dollars per year in damage to crops [21, 22]. Encompassing 15% of all identified nematode species, the most economically important species directly target plant roots of major production crops and prevent water and nutrient uptake resulting in reduced agronomic performance, overall quality and yields. Nematodes in the order Tylenchida are pathogens of plants, invertebrates, and fungi and are considered the most important agricultural pests [22].

Of all the important plant-parasitic nematodes, the most successful species are the sedentary groups which establish a permanent feeding site within the plant host and obtain nutrients while completing their lifecycles. Sedentary nematodes have a natural advantage over their migratory relatives due to a fascinating and complex method of host cell transformation resulting in the development a sustainable feeding structure. Interestingly, with over 4000 described plant-parasitic nematodes, only a small amount produce significant economic losses in crops. In a survey conducted on a variety of crops in the U.S, the major genera of phytoparasitic nematodes reported to cause crop losses were *Heterodera*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, and *Xiphinema* [23].

4.1. Wheat

Wheat (*Triticum aestivum*) is the most important cereal crop in the world. A staple food source for 40% of the world's population, approximately 758 million tons are produced globally [24]. Wheat yields are significantly decreased by the presence of cereal cyst nematodes (*Heterodera* spp.) in the *Heterodera avenae* group (*H. avenae*, *Heterodera filipjevi*, and *Heterodera latipons*) which also damage other important cereals including barley (*Hordeum vulgare*) and oat (*Avena sativa*). An estimated 3.4 million in profits are lost each year in U.S. wheat cultivating states Idaho, Oregon, and Washington [25]. In some wheat fields, the losses caused by *H. avenae* can range from 30 to 100% [26, 27]. In addition to cereal cyst nematodes, further losses of wheat are caused by root-lesion nematodes *Pratylenchus neglectus* and *Pratylenchus thornei*, and the seed gall or ear-cockle nematode, *Anguina tritici*. An inverse relationship between *H. avenae* and *P. neglectus* was shown on *P. neglectus* resistant and susceptible wheat cultivars infested with *H. avenae* [28] where a reduction in *P. neglectus* population densities was observed on both wheat genotypes. *Anguina tritici* is often a vector for *Rathayibacter tritici*, a Gram-positive soil bacterium which associates with *Clavibacter tritici* causing seed gall [29].

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4.2. Rice

Rice (*Oryza sativa* L.) is a staple food crop for most of the world's population with an estimated 480 million tons currently produced [30]. Plant-parasitic nematodes rank as one of the most important soil borne pests of rice and may account for annual yield losses of 10–25% worldwide. Over 100 species of nematodes affect rice production. *Meloidogyne* spp. is distributed worldwide and are significant pathogens of rice and other crops cultivated in temperate and tropical areas [31]. One of the most important species of *Meloidogyne*, is *M. graminicola*, may reduce rice yields up to 80% [32]. Symptoms of infection manifests as hook shaped galls, stunting, decreased tiller numbers and poor growth and reproduction [33]. The rice root-nematode *Hirschmanniella oryzae*, i.e., rice root nematode (RRN), is commonly found in irrigated rice production systems [34]. Widely distributed, *H. oryzae* has been reported in Asian countries such as India, Pakistan, Bangladesh, Sri Lanka, Nepal, Thailand, Vietnam, Indonesia, the Philippines, China, Korea and Japan [35] and in the U.S states, Louisiana and Texas.

4.3. Maize

Maize (*Zea mays*) is grown largely throughout the world with three largest production in North America Asia and Europe [21]. Over 50 species that are known to parasitize corn in the globally however, the most devastating genera include the root knot nematodes, *Meloidogyne* spp., the root lesion nematodes, *Pratylenchus* spp. and the cyst nematodes, *Heterodera* spp. [21]. In the U.S., the most economically important species are the lesion nematodes (*Pratylenchus* spp.) and the corn cyst nematode (*Heterodera zeae*). In most cases, symptoms of infection caused by these nematodes include poor development and leaf chlorosis with minor galling [36]. The needle nematode *Longidorus breviannulatus* is associated with stunting in corn and may cause economic losses in yields up to 60% [37].

4.4. Potato

The potato (*Solanum tuberosum*) is a member of the Solanaceae family, and is considered the third most important crop in the world with total world potato production estimated at over 376 million tonnes in 2013 [38]. Cyst nematodes are prolific pathogens of potato causing dramatic losses in yields. *Globodera rostochiensis* and *Globodera pallida* originate from S. America and are known pests of other members of the Solanaceae family including tomatoes and woody nightshade [39]. These nematodes are classified as quarantine pests in a number of countries including the U.S. and an estimated £ 50m year in profits are lost each year in the U.K. [40]. Other major plant-parasitic nematodes of potato include root-knot nematodes (*Meloidogyne* spp.), and the stem nematode *Ditylenchus destructor*. Among the four species of root-knot nematodes that affect potato production in the U.S., the Columbian root-knot nematode (*Meloidogyne chitwoodii*) is considered the most important species [41]. In addition to potato, sweetpotato (Ipomoea *batatas* L. Lam) is a major host for *D. destructor*, up to 100% yield losses have occurred in major production regions including the top producer China [42, 43]

4.5. Sweetpotato

The sweetpotato [*Ipomoea*. *batatas* (L) LAM] has been regarded as a plant of great significance throughout human history. Its cultivation dates to the prehistoric era, and it has been grown continuously as a staple food source. Global productions of sweetpotato is estimated at 105 million metric tons [44]. Currently, the sixth most important food crop, sweetpotato production has improved the economic status for communities throughout the world particularly in developing nations where it ranks as the fifth most important crop [44]. Approximately 10.2% of sweetpotato yields are lost each year due to the presence of plant-parasitic nematodes [20]. Root-knot nematodes (RKNs) are significant pests of sweetpotato causing symptoms of infection which include: stunted plant growth, yellowing of leaves, abnormal flower production, and gall production on roots leading to decreased nutrient and water absorption and necrosis and cracking on fleshy storage roots. Due to the economic importance of the storage root, root cracking is a primary concern for producers. Successful sweetpotato root-knot nematode resistant breeding programs involve the determination of resistance genes. Nematode resistance is governed by genotype [45] and is primarily quantitative [46]; therefore, the identification of genetic markers associated with root-knot nematode resistance requires broad scale molecular studies.

4.6. Root-knot nematodes

In a recent survey, the top 10 most important genera of parasitic nematodes in molecular plant pathology were ranked based on scientific and economic importance [47]. Ranked at the top of the list are root-knot nematodes (*Meloidogyne* spp.). The root-knot nematode (*Meloidogyne* spp.) comprises over 100 species, with *Meloidogyne javanica*, *Meloidogyne arenaria*, *Meloidogyne hapla*, and *Meloidogyne incognita* representing the most devastating threat to agricultural crop production [48]. The *Meloidogyne* spp. are globally distributed, have enormous host range and develop dynamic disease complexes with fungal species and bacteria which may exacerbate disease incidences in cultivated plants. The lifecycle of *Meloidogyne* spp. involves four developmental stages including larval stage 1 (within the egg), larval stage 2 (migratory), larval stage juvenile 3 (sedentary), larval stage 4 (sedentary) and adult stage (sedentary). Under favorable environmental conditions, first stage moulting to J1 larval stage within the egg occurs resulting in hatching, with or without the presence of a chemical stimulus. Infective second-stage juveniles (J2s) are often attracted to root exudates and migrate to root tips where they infiltrate behind the root cap at the elongation zone. Root knot nematodes attenuate plant cells by stylet thrusting and secrete cell wall degrading enzymes to separate the middle lamella during intercellular migration through root cortex cells as they target the undifferentiated procambium cells of the vascular cylinder. During later stages in primary infection, dorsal gland activity increases to promote shuttling of secretory granules to the stylet where proteinaceous secretions are released in the development of the primary feeding site—the giant cell [49]. The multi-nucleated giant cell is a result of nematode-induced endoreduplication within the host cell in the absence of cytokinesis. Cellular ingrowths arise to sequester solutes from the xylem [50] further enhancing nutrient availability. J2 larvae moult into larval stage 3 (J3) during the initial intake of plant nutrients from giant cells. Additional moulting occurs

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resulting into the J4 and final adult stage. Further reproductive development in females results in the characteristic "apple" shape associated with the Greek nomenclature *Meloidogyne*. The lifecycle completes when eggs are released into the soil from the gelatinous egg matrix formed on epidermal root tissue. Root-knot nematode infection is typically characterized by stunted growth, wilting, root galling and abnormalities in root formation.

4.7. Cyst nematodes

Cyst forming nematodes, or cyst nematodes, (*Heterodera* and *Globodera* spp.) rank second to root-knot nematode in agricultural and economic importance. The biology of cyst nematodes is similar to that of root-knot nematodes where J2 larvae infect the host and develop to adult stages within host tissue. In contrast, to root-knot nematode reproduction where eggs are deposited into a gelatinous matrix on root systems, eggs produced by cyst nematodes are preserved within the body of the female and are protected after her death until hatching under favorable conditions. Cyst nematodes enter root tips and induce specialized feeding structures in the infected plant roots called syncytia via esophageal gland secretions released by the stylet [51]. These secretions promote cell wall degradation and protoplast fusion of numerous adjacent cells to form the syncytium [52]. In agriculture, the most significant cyst nematode species are the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*, the soybean cyst nematode (*Heterodera glycines*) and cereal cyst nematodes (CCNs) (including *Heterodera avenae* and *H. filipjevi*. In the U.S., losses due to *H. glycines* is estimated at 1.286 billion [53]. *Globodera pallida* originated in South America and is now widely distributed in 55 countries. Yield losses of potato due to *G. pallida* range from 50 to 80% in heavily infested soils [54]. Although the beet cyst nematode, *Heterodera schachtii*, is a primary pathogen of sugar beets, it can parasitize plant species in 23 different plant families with losses of 30% in the families of *Chenopodiaceae* [55, 56].

4.8. Lesion nematodes

Ranked third among the most damaging nematodes in agriculture [57], approximately 70 species of root-lesion nematodes (*Pratylenchus* spp.) are distributed worldwide with a host range of nearly 400 plant species [57]. Among *Pratylenchus* spp., *P. thornei* is associated with yield reductions in wheat by as much as 85% in Australia, 70% in Israel, 50% in Oregon and 37% in Mexico [58]. Lesion nematodes are migratory, feeding mainly in the root cortex and may enter vascular tissues obtaining nutrients. Infection typically results in lesion formation and necrosis on roots with aboveground symptoms of chlorosis as well as reductions in leaf number and size [58, 59]. Host tissue injury resulting from infection may represent areas for secondary infection from other pathogens. Recently two new species of root-lesion nematodes (*Pratylenchus. kumamotoensis*, *Pratylenchus. pseudocoffeae*) were identified in Korea by morphometric and molecular analyses of internal transcribed spacer (ITS) and ribosomal DNA [60].

4.9. Burrowing nematode

The burrowing nematode, *Radopholus similis* [(Cobb, 1893) Thorne, 1949] is a migratory plant parasitic nematode, listed as a quarantine plant pest worldwide [61]. Over 250 plant species

serve as hosts for *R. similis* where it causes severe economic losses in yields. *R. similis* damages banana, citrus, pepper, coffee and other agronomic and horticultural crops and is considered the most important phytopathogenic nematode in banana-growing areas [62]. Effective control of *R. similis* remains problematic worldwide, and effective approaches must be identified and implemented. *Radopholus* Calreticulin (CRT) is a Ca^{2+}-binding protein that plays key roles in parasitism and represents a candidate target for controlling *R. similis. R. similis* CRT (*Rs-CST*) is expressed in the esophageal, reproductive and gastrointestinal regions as well as the eggs. Using plant-mediated RNA interference, *Rs-CRT* expression was significantly inhibited in the nematodes, and enhanced resistance was demonstrated in transgenic tomato plants [63]. In a bioassay-based study, phenylphenalenones extracted from Musa spp. showed anti-nematode effects on *R. similis* which was demonstrated by nematode motility inhibition [64].

5. Nematode parasitism genes

Nematode parasitism is conferred by the actions of a variety of genes that are upregulated during host infection. In an earlier review, a comprehensive discussion highlighted the structure, origin and functions of nematode parasitism genes and further supported the acquisition of parasitism genes through horizontal transfer from bacteria [49]. Since parasitism genes are usually required for infection, they represent important targets for the development of control measures. Parasitism genes often encode for effectors which are proteins or chemicals that elicit an immune response and/or trigger changes in the host cell architecture [51]. Recently two effector genes (*MhTTL2* and *Mh265*) were identified in the root-knot nematode *M. hapla* and were shown to be upregulated during primary infection [65]. *MhTTL2* encodes for a secreted protein bearing a transthyretin-like protein domain and is expressed in the amphids, with a potential role in the nervous system while *Mh265* is expressed in subventral glands. Nematode effectors including expansin, β-1,4-endoglucanase and polygalacturonase are released during primary infection and feeding site development. In plants, expansin proteins are secreted during growth processes to allow for cell enlargement [66]. Nematodes are believed to cause differential expressions of plant genes encoding cell wall modifying proteins including expansins [67] quite possibly to mimic endogenous expansin production during feeding site development. HaEXPB2, a predicted expansin-like protein found in cereal cyst nematode *Heterodera avenae* was associated with cell death in tobacco plants [68]. During primary infection of tobacco, *HaEXPB2* gene expression was localized in subventral glands of J2 nematodes and was later found in the cell wall. Silencing of *HaEXPB2* by RNA interference was associated with reduced nematode infectivity. Transcriptome sequencing analyses of early stage *H. avenae* juveniles has revealed a variety of potential effectors including plant cell wall-modifying proteins and homologues of secreted proteins involved in the detoxification of reactive oxygen species (ROS) including: peroxiredoxin, glutathione peroxidase, glutathione-S-transferase [69]. ROS release is associated with onset of plant defense signaling. New evidence suggests that root-knot nematodes may utilize plant peroxidase to reduce ROS levels and parasitize plants bearing the *Mi-1* root-knot nematode resistance gene [70]. In plants, pathogens may trigger a hypersensitive response which involves programmed

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cell death (a form of apoptosis) in the site of infection to prevent pathogen colonization. Apoptosis regulator BAX (BCL-2 protein 4) is a member of the Bcl-2 family of proteins found in plants and animals [71]. Two secretory effector candidate genes (No. 5, No. 100) identified by transcriptome profiling in *Meloidogyne enterolobii* suppressed BAX-induced programmed cell death suggesting their roles as plant immune modulators for nematode infection [72]. The SPRY (SPla and the RYanodine Receptor) protein domain is most likely a scaffold for mediating protein-protein interactions [73]. SPRY effectors from *Globedera* spp. was shown to suppress the plant defense responses [74].

6. Molecular basis of nematode resistance

The development of a resistance response may encompass a variety of physiological outcomes including: minor or complete absence of galling, differences in the degree of necrosis, the inability of the nematode to establish a permanent feeding site, and a decrease in female fecundity or egg output. To date, the majority of plant-parasitic nematode resistance genes bear the characteristic NBS-LRR (Nucleotide binding site—Leucine Rich Repeat) domains. These include the *Mi-1* gene from *Solanum peruvianum* (formerly *Lycopersicon peruvianum*) [75], *Hs1*$^{pro\text{-}1}$ from sugar beet [76] and *Gpa2* and *Gro1-4* from potato [77, 78].

Resistance to *Meloidogyne* in commercial resistant tomato cultivars (*Lycopersicon esculentum*) was originally identified in its wild relative *L. peruvianum* Mill. [79] followed by introgression of resistance into commercial breeding lines through backcrossing [80]. Several root-knot resistance gene homologues have been identified in tomato. *Mi-1.2* (referred to as *Mi-1*) confers resistance to multiple species of root-knot nematodes, [75] the potato aphid, *Macrosiphum euphoribiae* [81] and the whitefly, *Bemisia tabaci* [82]. *Rme1* is considered a potential component of the *Mi-1*-mediated signaling pathway as studies have indicated tomato *Rme1* mutants lack resistance to nematodes and whiteflies [66]. Molecular changes in *Rme1* protein conformation due to the presence of pathogens, may be recognized by *Mi-1.1* which signals the hypersensitive response in the "guard hypothesis" [83]. In carrots, inherited dominance of two root-knot nematode resistance genes *Mj1* [84] and *Mj2* [85] conferred resistance to *M. javanica*. The *RMIa* gene located in a subtelomeric position 300 kb physical distance between AMPP117 and AMPP116 markers and is associated with *M. incognita* resistance in peach (*Prunus* spp.) [86]. The Myrobalam plum (*Prunus cerasifera*) harbors dominant alleles (*Ma1*, *Ma2*, and *Ma3*) of a single gene *Ma*, a TIR-NBS-LRR class resistance gene, which confers broad spectrum resistance to multiple *Meloidogyne* spp. [87]. Using polymorphic sequencing analyses and genetic linkage mapping (RFLP, SSR) the *Ma* loci was precisely identified in the Myrobalan plum linkage group 7, while in a Japanese plum variety, a *Rjap* gene was localized at the same position in co-segregation with SSR markers previously associated with root-knot nematode resistance [88]. In sweetpotato, 275 candidate resistance gene analogs have been identified by degenerate PCR and molecular mining [89]. Plant-parasitic nematodes have been shown to manipulate host gene expression, therefore the identification of differential expression patterns of transcript levels for defense-related genes is a critical component

in the determination of molecular factors of root-knot nematode resistance. Traditional identification of root-knot nematode resistance has involved the use of bulk segregant analysis [90] to map out qualitative traits between pooled plant genomes. Bulk segregant analysis has been used in tandem with random amplified polymorphic DNA assays to identify molecular markers at specific loci associated with root knot resistance in sweetpotato where genotypes are often isogenic [91]. Polymorphic events in resistance genes that confer effector recognition has been demonstrated in *Arabidopsis* resulting in a bifurcation that distinguishes resistant and susceptible allele clades [92].

Genome-wide expression profiling analyses using next-generation sequencing technologies are often employed in the analysis of host-nematode interactions. The resultant data from global transcriptome assays are used to target genetic traits associated with plant immune responses in response to various pathogens and to distinguish plant genotypes for resistance or susceptibility to certain diseases. Our general understanding of discreet molecular events involved in compatible (susceptible) and incompatible (resistant) plant-nematode interactions is limited in comparison to other significant host-pathogen associations. Recently, RNA-Sequencing has been frequently used in plant pathological studies to profile gene expression patterns in host plants and pathogens [93, 94]. Differential genetic expression profiles of many specific genes involved in plant immune responses has been shown in resistant and susceptible plants challenged by root-knot nematodes [48, 95]. The identification of novel defense-related transcripts and the elucidation of pathways involved in plant immune responses to nematodes have been recorded for important economic crops including cotton [96], rice [97], and soybean [98]. Transcriptome profiling of resistant and susceptible tobacco varieties infected with root-knot nematodes has shown differential expression patterns among genes involved in cell wall modification, auxin production and oxidative stress [99].

6.1. Plant immune responses

Due to their immobile lifestyle, plants have developed sophisticated molecular strategies to prevent pathogen invasion [100]. Plant defense has been characterized as a two- prong approach. In incompatible (resistant) plant-pathogen interactions, the presence of microbial/pathogen/-associated molecular patterns (M/PAMPs) including: toxins, glycoproteins, carbohydrates, fatty acids and proteins can trigger the upregulation of a network of host genes and corresponding proteins involved in an innate response termed pathogen-triggered immunity (PTI). Plant pathogens have evolved specialized effector molecules to suppress this first line of defense leading to effector-trigged susceptibility (ETS). In turn, plants have developed resistance genes which recognize specific effectors triggering a more robust defense response characterized as effector-triggered immunity (ETI). A hallmark of ETI is a hypersensitive cell death response (HR) at the infection site which prevents pathogen colonization [101].

6.2. Reactive oxygen species and antioxidant production

During plant metabolic processes, the accumulation of reactive oxygen species (ROS) by-products including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$)

and hydroxyl radicals ($^{-}$OH) is often continuous, as these highly reactive molecules are localized to various cellular compartments. ROS are primarily generated by NADPH oxidases and superoxide dismutases and production is associated with numerous abiotic and biotic stress responses. Activation of ROS was shown to be critical during the defense response to root-knot nematode invasion [102]. ROS accumulation is toxic to nematodes and can often lead to induced oxidative destruction of infected cells during the hypersensitive response, to prohibit pathogen colonization. Increased ROS production is often correlated with the activation of antioxidant gene expression. These oxidative/reduction reactions must be tightly regulated to eliminate inadvertent plant tissue damage. Antioxidant enzymes including peroxidases are primarily responsible for the maintenance of a steady-state ROS level however, certain classes of peroxidases act as producers of ROS depending on the cyclic (catalytic or the hydroxylic) nature of the enzyme. ROS- producing Class III peroxidase genes were upregulated during an incompatible reaction in *H. avenae*-resistant wheat cultivars [103]. Peroxidase reduces H_2O_2 levels via H_2O_2-dependent polymerization of hydroxycinnamyl alcohols which promotes defense responses including lignin synthesis and cell-wall reinforcement by the cross-linkage of cell wall proteins [104]. Higher induction of peroxidase groups was observed in resistant plant species during *H. avenae* and *M. incognita* infection [105].

6.3. Pathogenesis-related proteins

Presently, 17 families of pathogenesis-related (PR) proteins have been identified based primarily on their enzyme function, activity and amino acid sequence homologies [106]. The PR family are characterized as plant allergens inclusive of an assortment of proteins such as: b-1,3 glucanases, chitinases, proteinase inhibitors, defensins, ribonucleases and thionins. PR gene expression is often induced by ethylene, salicylic acid, jasmonic acid, xylanase, and systemin signaling pathways. The molecular functions of PR proteins are often species specific with great diversity in the mode of action and structure between protein groups. Most PRs possess antifungal, antiviral, antibacterial and insecticidal activity and are primarily involved in plant developmental processes and environmental stress responses. PR proteins were initially reported in tobacco leaves during a hypersensitive response to the tobacco mosaic virus (TMV) [107, 108] and have been induced in response a wide variety of pathogens including nematodes. During nematode infections, PR transcripts may accumulate in high concentrations and are associated with the long distance immune response termed systemic acquired resistance (SAR) [109]. Increased expression of *PR-1*(P4) transcripts was observed at 3 days' post-infection in the *G. rostochiensis*-infected resistant plants compared with the uninoculated controls [110].

6.4. Callose deposition

Cell wall modifications often occur during plant-pathogen interactions which are demonstrated by the deposition of cell wall appositions leading to the development of papillae. Structural components associated with papillae formation are: callose, phenolics including lignin, phenolic conjugates such as phenolic–polyamines, reactive oxygen species, peroxidases, cell wall structural proteins (arabinogalactan proteins and hydroxyproline-rich glycoproteins) and cell wall polymers (pectin and xyloglucans). Callose (beta-1-3-glucan) deposition,

lignification and suberization are plant developmental processes further associated with the restriction of systemic pathogen movement during PTI. Defense-associated cell wall strengthening through lignin and callose synthesis is signaled by cell wall degradation in a feedback mechanism which occurs in response to pathogens [111]. In addition to promoting declines in localized microbial populations, callose deposition also prevents the translocation of PTI-suppressive effectors. Interestingly, the cuticular chitin derivatives of plant-parasitic nematodes may activate the innate immune response. Although the cuticle is generally believed to be devoid of chitin, it is possible that chitin derivatives or chitin previously deposited in the stylet are recognized by the host which activates callose deposition at the site of penetration. The overexpression of the ethylene response transcription factor RAP2.6 in *Arabidopsis* enhanced plant basal resistance to *H. schachtii* [112]. Increased expression of jasmonic acid-related genes and callose deposition were observed at nematode infection sites.

7. WRKY transcription factors

WRKY transcription factors are transcriptional regulators of many developmental processes in plants and are associated with abiotic and biotic stress responses. The WRKY domain is almost exclusive to plants characterized by a highly-conserved core WRKYGQK motif and a zinc finger region. The critical role of WRKY transcription factors (WRKY TFs) in plant defense responses has been well documented [113, 114]. Their ability to bind to pathogen responsive cis acting W-box promoter elements in *PR1* genes is indicative of their role in plant immunity [113]. *Arabidopsis WRKY72* was reported to have a significant contribution to *Mi-1*-mediated defense against RKNs, potato aphids [114] and oomycete pathogen *Hyaloperonospora arabidopsidis* the causal agent of downy mildew [115]. *WRKY* gene expression is altered during plant-parasitic nematode interactions. The development of cyst nematode *H. schachtii* feeding site (syncytia) involves the up-regulation of WRKY23 [116]. Conversely, endogenous *WRKY33* gene expression levels were strongly downregulated in syncytia formed in *Arabidopsis* roots, while plants overexpressing *WRKY33* showed a 20–30% reduction in the presence of female nematodes [117] a possible indication of its role in plant defense.

7.1. Calreticulin proteins

In animals, endoplasmic reticulum (ER) localized calreticulin proteins are integral components in calcium homeostasis as well as protein folding and are involved in other significant cellular functions [118]. Ubiquitously expressed in plants, calreticulin performs similar functions to its animal counterpart despite 50% differences in amino acid sequence homology. Plant calreticulin is described as a molecular calcium-binding chaperone that promotes protein folding, calcium signaling and homeostasis, and oligomeric assembly in a calreticulin/calnexin cycle. Calreticulin may interact with a majority of monoglucosylated glycoproteins synthesized in the ER, while certain isoforms have been associated with the expression and quality control of the elongation factor Tu receptor-like protein kinase (EFR) [119] an important event in M/PAMP-triggered immune responses. The significance of calreticulin

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isoform-3 (*AtCRT-3*) function through gene deletion was identified in *Arabidopsis* plants [120]. Plant transformants with repressed *AtCRT*-3 gene activity were impaired in perception of M/PAMP-associated efl-18 and deficient in EFR protein expression and anthocyanin content. Furthermore, they concluded that AtCRT-3 may be involved in the unfolding and activation of EFR based on its primary molecular function and recognition of EFR N-glycosyl binding sites. Recently, studies have shown that root-knot nematodes secrete calreticulin, which plays an important role in infection [121].

7.2. Plant proteinase inhibitors

Plants utilize an arsenal of defensive mechanisms to evade infection from nematodes. One important strategy involves limiting nematode feeding capabilities. Plant proteinase inhibitors are involved in many physiological processes including protein turnover and proteolysis during metabolism however; other evidence has supported an alternative role in defense against plant pathogens [122]. Plant proteinase inhibitors degrade nematode proteases preventing the breakdown of food material which reduces nutrient absorption in the nematode. As early as 1947, the idea of proteinaceous protease inhibitors was formulated as Mickel and Standish observed differences in larval development on soybean cultivars [123]. The applicability of proteinase-inhibitors in nematode resistance was initially demonstrated in transgenic potato expressing a serine proteinase-inhibitor cowpea trypsin inhibitor (CpTI) [124]. CpTI expression directly influenced the sexual fate of *G. pallida* toward a higher ratio of smaller males with reduced damage observed on roots. Out of the four major classes of plant proteinases inhibitors (cysteine, serine, aspartic, metallo-proteinases) cysteine and serine proteinase inhibitors have gained considerable interest as effective defense molecules nematodes due to their specificity in the degradation of the major digestive enzymes (proteases) in plant-parasitic nematodes [125].The effectiveness of proteinase inhibitors can be attributed to its small size, which benefits its inclusion with nutrient molecules absorbed by some plant-parasitic nematodes. In tomato, overexpression of phytocystatin gene, *CeCPI* isolated from taro (*Colocasia esculenta*) showed enhanced resistance to root-knot nematodes demonstrated by reduced galling and an influence on sex determination [126]. In sweetpotato, sporamin which is classified as a Kunitz-type trypsin inhibitor, accounts for 60–80% of total soluble protein. Sporamin is constitutively expressed in the tuberous root in comparison to in the stem or leaves and is expressed systemically in response to wounding and other abiotic stresses [127]. In previous studies, three forms of sweetpotato sporamin showed strong trypsin inhibitory activity *invitro* [128]. Additional research has resulted in the identification of sporamin-mediated resistance to cyst nematodes [129]. Decreased nematode development correlated with trypsin inhibitor activity of sporamin which was the critical factor for inhibition of growth and development of cyst nematodes on sugar beet roots. Plant genotypes that produce high sporamin levels may have a selective advantage in defense to plant-parasitic nematodes.

7.3. Plant hormones

The roles of plant developmental hormones, ethylene, jasmonic acid and salicylic acid have been well established during plant immunity [130, 131]. Jasmonic acid (JA) and ethylene (ET)

signaling pathways work synergistically while the salicylic acid (SA) pathway is antagonistic to JA/ET pathways [132]. In a prior study, exogenous ethyhlene (ethephon) and jasmonic acid (methyl jasmonate) application triggered the induction of PR proteins and the activation of systemic defense against root-knot nematodes on rice [133] These findings suggest a critical role of an active intact jasmonic acid pathway during the activation of systemically induced resistance. The combination of exogenous jasmonic acid and biogenic elicitor arachidonic acid, decreased galling on tomato roots two-fold in comparison to controls [134]. The role of salicylic acid has been well documented in the efficacy of host resistance to root-knot nematodes. Pathogenesis-related protein expression was associated with salicylic acid-dependent systemic required resistance in tomatoes pretreated with salicylic acid under root-knot nematode challenge [109]. Expression of a *NahG* which encodes for an enzyme that degrades salicylic acid to catechol, reduced *Mi-1* gene- mediated root-knot nematode resistance in transgenic tomatoes [135].

8. Disease management of plant-parasitic nematodes

8.1. Cultural control

For many years, crop rotation and cover cropping are often utilized in integrated pest management protocols to reduce plant-parasitic nematode incidence and replenish soil nutrient levels. Soil nematode levels have been effectively decreased by rotational cultivation of non-host cultivars however, the wide host range of *Meloidogyne spp*. often diminishes the effectiveness of crop rotation [136]. Planting corn as a rotational crop has been shown to reduce northern root-knot nematode (*M. hapla*) incidence however; population densities of other *Meloidogyne spp*. may increase with persistent cultivation. Plant species with resistance to mixed *Meloidogyne* populations have been identified. Leguminous cover crops *Mucuna pruriens* L., and *Crotalaria spectabilis* showed multiple resistance to three species of root-knot nematodes (*Meloidogyne arenaria, M. incognita*, *M. javanica*) [137]. In certain cases, the very nature of crop production may suppress the magnitude of infection. Rice is cultivated under flooding conditions which does not favor the nematode lifestyle. In Taiwan, crop rotations with rice or taro combined with cultural control methods including flooding and bare fallowing was shown to decrease nematode soil populations and increase strawberry yields [138].

8.2. Plant extracts

Plant extracts often contain a myriad of compounds which demonstrate nematode suppressive properties. Ethanolic extracts of *Azadirachta indica* (neem), *Withania somnifera* (ashwagandha), *Tagetes erecta* (marigold) and *Eucalyptus citriodora* (eucalyptus) were reported to show nematicidial activity against *Meloidogyne incognita*, *Helicotylenchus multicinctus* and *Hoplolaimus* which was comparable to chemical nematicide controls [139]. In other reports, increased plant growth and development were shown in plants propagated with the addition of a variety of extracts. Root-knot nematode egg hatch and larval development was dramatically reduced by leaf extracts from *Hunteria umbellata* and *Mallotus oppositifolius* which coincided with

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increased growth of cashew seedlings [140]. Plant height, fruit production and weights of *M. incognita*-infected tomato were significantly increased by the addition of ethanol extracts from *Azadirachta indica* leaves, *Capsicum annuum* fruits, *Zingiber officinale* rhizomes and *Parkia biglobosa* seeds in comparison to non-treated controls [141].

8.3. Biological control

With increasing demands in organic agriculture and concerns for environmental welfare, the use of chemical pesticides has decreased. Alternative means of pest management such as the use of biological controls are of great interest for crop producers. The efficacy of nematophagous bacteria and fungi in the control some nematode pests, including cyst and root-knot nematodes has been well-documented [142, 143]. Parasitic bacteria of *Pasteuria spp.* have been reported to infect 323 nematode species including both plant-parasitic nematodes and free-living nematodes [144]. Three methods of application for *P. penetrans* were evaluated for nematode control including seed, transplant, and post-plant treatments [145]. In greenhouse studies involving cucumber, all three *Pasteuria* treatments were shown to reduce galling caused by *M. incognita* as well as soil nematode numbers and nematode reproduction. In other reports, *M. incognita* suppression was observed in field soil treated with *P. penetrans* in comparison to untreated soil [146]. Other genera of bacteria including *Bacillus* spp. have shown great promise in nematode management. *B. cereus* strain S2 treatment resulted in a mortality of 90.96% to *M. incognita* [147]. *B. firmus* YBf-10 exhibited nematicidal activity against *M. incognita*, which was clearly demonstrated by an inhibition of egg hatch and motility [148]. Nematophagous fungi *Pochonia chlamydosporia* has potential as a biological control agent for *M. incognita* in vegetable crops. Along with crop rotational methods, *P. chlamydosporia* was shown to reduce nematode levels in soil previously used for root-knot nematode susceptible tomato [149]. Nematophagous fungal products including chitinases show great potential for the development of biopesticides. Certain root-knot nematode species have transparent protective chitin-containing shells. Purified chitinase LPCHI1 from *Lecanicillium psalliotae* was shown to degrade *M. incognita* eggs [150].

8.4. Host resistance

Chemical nematicides are often used in the management of root-knot nematodes however; EPA restrictions in some soil fumigants due to increased environmental toxicity coupled with the expensive costs associated new nematicide development limit their availability. The very nature of these mammalian pesticides poses a significant risk to humans. Plant-parasitic nematodes often reside in plant tissue which makes soil delivery applications of the chemical challenging. The incorporation of plant varieties that harbor multiple resistance to an array of plant pathogens is an attractive and practical approach for plant breeders. However, the conserved use of specific genotypes of disease resistant cultivars may contribute to increased pathogen aggressiveness resulting in epiphytotic conditions; therefore the identification of additional resistant varieties becomes increasingly necessary for long term control. For many years crops have been artificially selected for their inherent disease-resistant properties through phenotypic screenings and genetic analyses. Nematode-resistant genes found

in gene pools of a variety of plant species have been introgressed into the genomes of economically important crops with natural susceptibility through transgenic technologies such as agrobacterium-mediated transformation [151, 152].

Plants synthesize and release an array of volatile organic compounds in response to damage. Plant terpenes/terpenoids are secondary metabolites produced by terpene synthases in plants and are involved plant survival and biotic and abiotic stress responses. Functional characterization of one member of the soybean TPS gene family, designated GmAFS suggested an anti-nematode role [153]. Transgenic hairy roots overexpressing GmAFS were generated in an *H. avenae*-susceptible soybean line. Plants showed significantly higher resistance to *H. avenae* burden than controls.

RNA interference (RNAi) is a method of gene silencing observed in a wide range of organisms. This method of gene silencing has become a useful tool for biologists to study biological processes and has been developed into a novel control strategy for engineering plants with nematode resistance. First identified in plants [154] the mechanism of action was elucidated in the nematode model organism *C. elegans* [155]. RNAi involves the suppression of specific transcripts to minimum expression levels as a method of post-transcriptional gene silencing during developmental processes and is believed to be a response to double-stranded viral entry. RNAi is premised on the cell's ability to recognize and degrade double-stranded RNA (dsRNA). The dsRNA is processed into small interfering RNA (siRNA) by the enzyme Dicer, a ribosome III-like enzyme. Double-stranded siRNA is unwound into two single-stranded RNAs and one strand serves as a guide which associates with the RNA-induced silencing complex (RISC). This complex associate with the specific complementary mRNA expressed in the cell where the RNAse H enzyme Argonaute degrades the mRNA resulting in gene silencing. Since the discovery of RNA-interference, researchers have developed transgenic constructs that specifically target genes for functional characterizations. More recently, plants have been engineered to expresses double-stranded RNA that silence important genes in plant-parasitic nematodes [156, 157]. As nematodes feed on the plant cytoplasm, the uptake of the siRNA triggers the endogenous RNAi mechanism within the nematode, silencing the target gene involved in infection [158]. The RNAi approach was applied, using sequence fragments from *M. incognita* genes that encode for two heat-shock protein 90 (HSP90) and isocitrate lyase (ICL). Heterologous expression of RNAi constructs in tobacco plants correlated to a significant level of resistance against *M. incognita*. Delayed galling and decreased egg production was observed in plants expressing HSP90 dsRNA. The *16D10* effector gene encodes for a secretory peptide synthesized in the subventral esophageal glands of root-knot nematodes which plays an important role in giant cell formation cells [156]. *In planta* expression of 16D10 dsRNA in Arabidopsis conferred in resistance effective against the four major root-knot nematode species [156]. In transgenic lines of potato expressing a 16D10 RNAi construct (Mc16D10L), the number of *M. chitwoodi* egg masses and eggs was significantly decreased in comparison to empty vector controls [159]. *Mc16D10L* expression was reduced in eggs and juveniles developed on transgenic potato which suggest a stable heritability of the construct. Decreased egg production was also observed in transgenic grape lines expressing *16D10L* [160].

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The use of site-specific DNA endonucleases including Zinc finger nucleases (ZFNs), [161] transcription activator-like effector nucleases (TALENs) [162] and now clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 [163] have equipped researchers with the ability to specifically inactivate genes and target genetic regions for homologous recombination of input DNA. In general, double-stranded breaks introduced by nucleases activates DNA repair mechanisms which generate mutations in the target sequence conferring a loss of expression i.e., gene editing. Homologous recombination of exogenously supplied sequences can result in genetic modifications (knock-ins). CRISPR technology has important advantages over TALENS and ZFNs including; ease of use [164] target site selection [165] and overall efficiency, although off-target effects remains an important issue of concern [166]. CRISPR/Cas9 system may be used to alter the expression of resistance genes for constitutive expression against plant-parasitic nematodes. For example, point mutations in the *snc1* (suppressor of npr1-1, constitutive 1) locus in *Arabidopsis* plants resulted in constitutive expression of pathogenesis-related proteins and enhanced disease resistance against two plant pathogens [167]. The mutation was mapped to a single nucleotide change in 120-kb region on chromosome 4 which contains a cluster of resistance genes. In a recent sweetpotato study, putative disease resistance gene *DRL23* showed elevated expression in resistant sweetpotato genotypes when compared to susceptible plants at days 14 and 46 post-inoculation with *Meloidogyne incognita* inoculum [168]. To identify any polymorphisms in amino sequences between *DRL23* from resistant and susceptible cultivars, protein alignments using the NCBI BLAST (Basic Local Alignment Search Tool) was performed. Interestingly, variations in amino acid sequences occurred between resistant (positions 187–231) and susceptible (positions 57–102) which corresponded to the NBS domain. Mutations in the NB-ARC domain often abolish R-protein function, indicative of the functional relevance of this domain [169]. Precise targeting by CRISPR may be useful in restoring gene function by sequence replacement in defense-related genes thereby enhancing resistance to nematode infection.

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