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Nematode Pathogenesis and Resistance in Plants

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INTRODUCTION

Nematodes comprise a large phylum of animals that includes plant and animal parasites as well as many free-living species (Maggenti, 1981). Plant parasitic nematodes are obligate parasites, obtaining nutrition only from the cytoplasm of living plant cells. These tiny roundworms (generally ~1 mm long and barely visible to the human eye) damage food and fiber crops throughout the world and cause billions of dollars in losses annually (Sasser and Freckman, 1987). Some plant parasitic nematodes are ectoparasites, living outside their host. These species cause severe root damage and can be important virus vectors (Brown et al., 1995). Other species spend much of their lives inside roots as migratory or sedentary endoparasites. Migratory parasites move through the root, causing massive cellular necrosis. However, it is the sedentary endoparasites of the family Heteroderidae that cause the most economic damage worldwide. This group is the focus of the review.

The Heteroderidae can be divided into two groups: the cyst nematodes, which include the genera *Heterodera* and *Globodera*; and the root-knot nematodes (genus *Meloidogyne*). The soybean cyst nematode (*Heterodera glycines*) is the most economically important pathogen of soybean in the United States. Potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) cause losses in potato-growing areas worldwide (Ross, 1986). Root-knot nematodes, so-called for the characteristic root galls or root knots that they form on many hosts (Figure 1A), infect thousands of plant species and cause severe losses in yield of many crops throughout the world (Mai, 1985). Symptoms of diseased plants infected by these groups of nematodes include stunted growth, wilting, and susceptibility to other pathogens.

Nematodes in these three genera have complex interactions with their host plants that generally last more than a month and result in major morphological and developmental changes in both organisms. During the infection, elaborate developmental and morphological changes occur in host root cells, especially in those that become the feeding cells that provide the sole source of nutrients for the nematode. There has been a recent burst of activity in the investigation of the molecular changes that mediate the host–parasite interaction. This activity has been sparked by both the availability of new molecular tools and the urgent need to identify new approaches to control these pests since the loss of many effective pesticides. Recent reviews have discussed nematode-induced genes in host plants and strategies for engineering synthetic resistance to nematodes (Sijmons et al., 1994; Gheysen et al., 1996). We focus on new information in these areas and discuss the progress being made toward understanding natural host resistance. Recent investigation of nematode signals that trigger the molecular and developmental changes in plants that are associated with parasitism are also discussed.

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THE PARASITIC CYCLE

Cyst and root-knot nematodes spend most of their active lives within plant roots, feeding on dramatically modified host cells. Their life cycle involves passage through a series of four juvenile stages, separated by molts, during which the cuticle is replaced. The infective stage is the motile, second-stage juvenile that penetrates the root and migrates to a site near the vascular tissue to establish a permanent feeding site. After feeding is initiated, the nematode becomes sedentary and then undergoes three molts during development to the adult stage. Adult females are bulbous and nonmotile. Egg production begins at ~3 to 6 weeks after the initial infection, depending on the species and environmental conditions. Gender is determined epigenetically, with males increasing in frequency under conditions of crowding or poor nutrition (Triantaphyllou, 1973). Males also pass through a nonmotile developmental stage but regain motility during the third molt before leaving the root. Cyst nematode adult females are globular (*Globodera*) or lemon-shaped (*Heterodera*) and become filled with hundreds of embryonated eggs (Figure 1B). At the death of the female, her body wall forms a protective enclosure for the eggs, which can then remain viable in the soil for many years.

Cyst nematode infection of roots has been monitored in great detail by high-resolution video-enhanced microscopy (Wyss and Zunke, 1986). Second-stage juveniles enter roots through the epidermis and migrate through the cortex by piercing and rupturing cell walls with their stylet. This causes cellular damage and usually some necrosis (Golinowski et al., 1996). After

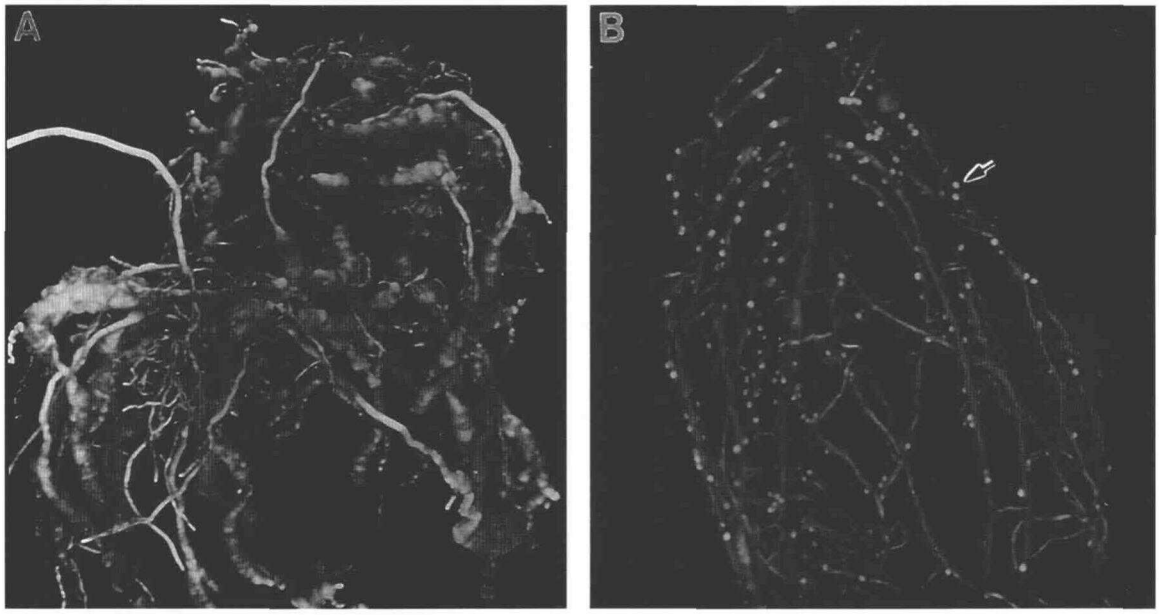

Figure 1. Nematode-Infected Root Systems.

(A) Tomato root system galled by root-knot nematodes. The swollen roots or galls are characteristic of this disease.
(B) Soybean root system infected with the soybean cyst nematode. White adult female nematodes (arrow) are visible on the root surface.

penetrating the endodermis, the nematodes pierce the wall of a procambial cell near the primary xylem and inject secretions. The injected cell rapidly begins to develop into a feeding site, a metabolically active cell in which the cytoplasm expands and becomes dense. Walls to neighboring cells partially dissolve, and the protoplasts fuse, resulting in a progressively larger syncytium that may incorporate >200 cells. Ultimately, syncytia are characterized by enlarged nuclei with large nucleoli, dense cytoplasm, pronounced cytoplasmic streaming, and cell walls with elaborate ingrowths on faces abutting vascular tissue (Jones, 1981).

Root-knot nematode behavior during the early stages of the infection process, which has also been monitored by video microscopy inside transparent Arabidopsis roots, differs in some important aspects from that of cyst nematodes (Wyss et al., 1992). The second-stage juveniles are attracted to the zone of elongation, where they penetrate the root and then migrate intercellularly, separating cells at the middle lamella in the cortical tissue. This process appears to include both mechanical force and enzymatic secretions from the nematode. In Arabidopsis, the infective juveniles usually migrate down to the root tip, then turn around in the region of the root apical meristem. They then migrate up the center of the root to the zone of differentiation. Here, in response to signals from the nematode, procambial cells adjacent to the head of the nematode develop into "giant cells." These large, multinucleate, metabolically active cells serve as a permanent source of nutrients for the endoparasite (Huang, 1985; Figures 2A and 2B). Each nematode triggers the development of five to seven giant cells, each containing as many as 100 enlarged, highly lobed nuclei. Giant cells have dense cytoplasm and thickened walls remodeled to form elaborate ingrowths, much like syncytial cells formed by cyst nematodes. The multiple nuclei in giant cells result from mitosis uncoupled from cytokinesis. In addition, individual nuclei have a high DNA content (Wiggers et al., 1990), indicating that endoreduplication has occurred. Concurrent swelling and division of cortical cells around the nematode lead to the formation of the galls and the distorted root structure characteristic of *Meloidogyne* spp infection (Figures 1A and 2). After development of the female, which usually takes ~3 weeks, eggs are released on the root surface in a protective, gelatinous matrix.

Although the molecular signals for the development and maintenance of giant cells have not been identified, it has been demonstrated that the maintenance of their integrity is dependent on continuous stimulation by the nematode (Bird, 1962). Root-knot nematodes obtain nutrients symplastically from the phloem via the giant cells (Dorhout et al., 1993) and cause a strong nutrient sink effect on the plant (McClure, 1977).

Induction of the discrete feeding sites produced by cyst and root-knot nematodes undoubtedly involves different mechanisms. Cell fusions after cell wall dissolution give rise to syncytia, whereas abnormal cell growth after nuclear division creates the giant cells. How nematodes dramatically alter root cell development to produce and maintain giant cells or syncytia is a question of great interest.

NEMATODE SPECIALIZATIONS FOR PARASITISM

The keys to understanding the nematode signals that trigger the complex plant response to infection lie in the biology of the nematode. Nematodes have complex behavior regarding host perception and parasitism. As is typical of the phylum, they possess a central nervous system and complex chemosensory organs called amphids (Figure 3). Chemosensory signals appear to be important for nematode attraction to host roots and also for the identification of appropriate sites for penetration of the host and initiation of feeding. However, little is known about the nature of these plant stimuli (Perry, 1996).

Plant parasitic nematodes possess two specialized structures, stylets and esophageal secretory glands, that are thought to be essential for many aspects of parasitism (Hussey, 1989; Hussey and Mims, 1990). The stylet is a hollow, protrusible structure at the anterior end of the nematode that is used to pierce plant cell walls (Figure 3). Heteroderidae possess three esophageal secretory glands. Each gland is a single, large cell that is connected to the nematode's esophageal lumen through an elaborate valve. Cyst and root-knot nematodes establish a prolonged biotrophic feeding association with the feeding cells, feeding periodically from the syncytial cell or from each of the giant cells. Secretions from the esophageal glands are released through the stylet. These secretions are thought to contain the biochemical trigger(s) for giant cell and syncytium development as well as substances important for the initial penetration and migration. During feeding, the stylet is inserted through the cell wall without piercing the plasma membrane, which becomes invaginated around the stylet. The nematode withdraws nutrients from the cytosol of the parasitized cell through a minute hole created in the plasma membrane at the stylet orifice (Figure 3). Callose accumulates between the plasma membrane and cell wall around the stylet (Hussey et al., 1992). However, this response does not appear to inhibit nematode feeding, presumably because callose deposition is absent where the plasma membrane is tightly appressed against the stylet orifice.

During nematode feeding, a structure called a feeding tube, associated with the stylet, is found in the cytoplasm of the host feeding cell (Hussey and Mims, 1991). A new tube, which appears to be of nematode origin (Hussey et al., 1994), is formed each time the nematode reinserts its stylet into a feeding cell, resulting in the presence of numerous feeding tubes in giant cells or syncytia. In giant cells, a portion of the endomembrane system rearranges to produce a compact membrane system

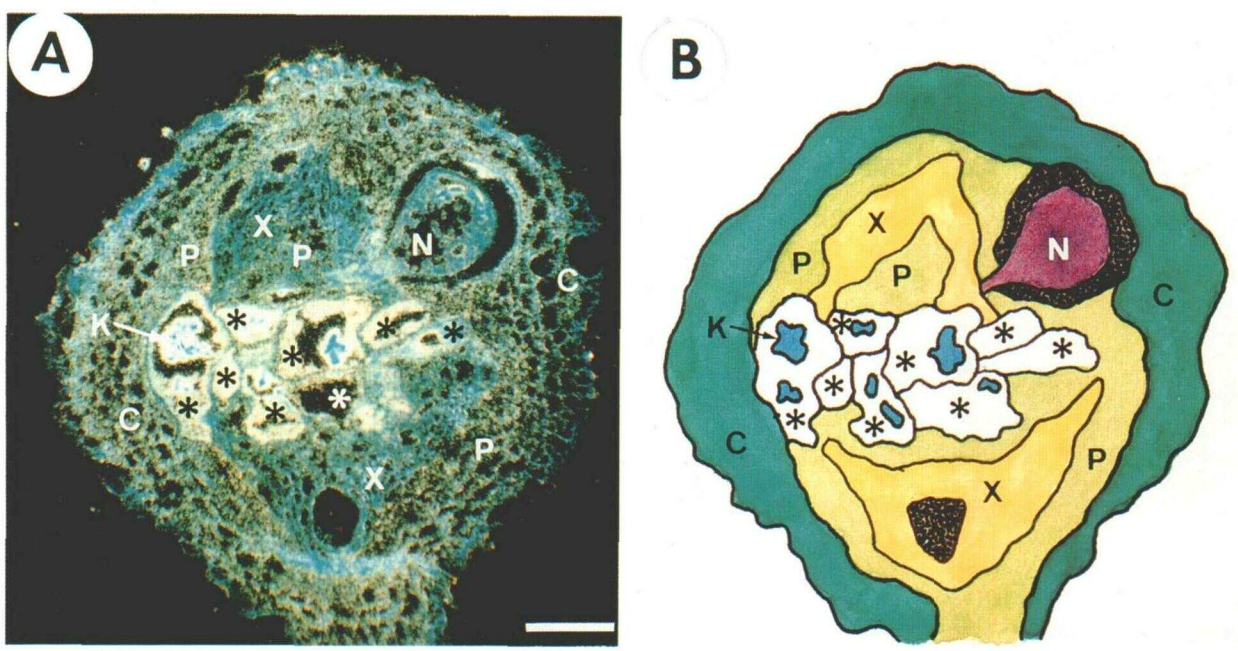

Figure 2. Cross-Section of a 4-Week-Old Gall Induced by *M. incognita* on Tomato Roots.

(A) In situ hybridization, using probe *Lemmi9*, a gene expressed in giant cells. This is a dark-field micrograph, and light-colored dots represent the *Lemmi9* hybridization signal. Bar = 200 μm.

(B) Schematic drawing of the gall in **(A)**.

This figure was reproduced from Van der Eycken et al. (1996) with permission of Blackwell Science Ltd. C, cortex; K, clustered nuclei; N, nematode; P, parenchymatic gall cells; X, xylem; *, giant cell.

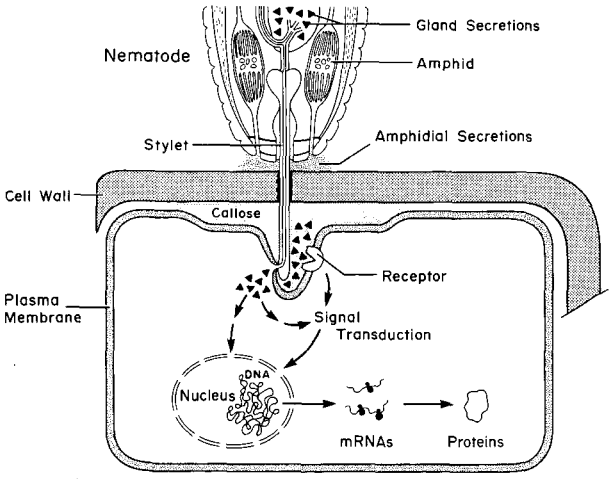

Figure 3. Schematic Model of Interactions of a Nematode with Its Feeding Cell.

The anterior end of the nematode is shown with the stylet protruded through the plant cell wall. Gland secretions (▲) originating from the esophageal glands of the nematode may be deposited outside the plasma membrane and interact with a membrane receptor or injected directly into the cytoplasm of the recipient cell through a perforation in the plasma membrane at the stylet orifice. Secretions from the amphids, chemosensory organs of the nematode, collect at the cell wall surface and may also have a role in the interaction.

around the feeding tube. The intimate association of the membrane system with the feeding tube suggests that it might function in transporting nutrients to the feeding tube for withdrawal by the parasite (Hussey and Mims, 1991). One possibility is that these structures act as molecular sieves during food ingestion. Microinjection experiments with fluorescently labeled dextrans determined that dextran uptake by *H. schachtii* in Arabidopsis has a size exclusion between 20 and 40 kD (Bockenhoff and Grundler, 1994).

Identification of esophageal gland secretory products is currently an intense area of investigation for both cyst and root-knot nematodes (Hussey, 1989; Hussey et al., 1994). Analyses of stylet secretion composition have shown the presence of proteins and carbohydrates but not nucleic acids (Hussey, 1989). Enzyme activities, including cellulase (Bird et al., 1974) and proteinase (Koritsas and Atkinson, 1994), have been detected in exudates from nematodes, but more detailed investigation has been hampered by the tiny amount of material available. Several monoclonal antibodies specific for secretory glands and/or stylet secretions have now been identified (Atkinson and Harris, 1989; Davis et al., 1994; Goverse et al., 1994; Hussey et al., 1994; de Boer et al., 1996), and studies with these antibodies have provided information on the subcellular localization and temporal synthesis of specific secretory components. Monoclonal antibodies have also been used to identify and purify secretory proteins as well as to isolate genes expressed in esophageal glands (Ray et al., 1994). As genes encoding nematode secretory proteins are cloned and characterized, determining their role in triggering the plant response will become more feasible.

MOLECULAR RESPONSES OF THE HOST

The complex morphological and physiological changes that occur during the establishment of feeding sites are reflected by altered gene expression in the host (reviewed in Sijmons, 1993; Atkinson, 1994; Niebel et al., 1994; Sijmons et al., 1994). Molecular responses include those to wounding or stress caused by nematode infection as well as perturbations directed toward the initiation and maintenance of feeding sites. Because many of the genes identified in the response are members of gene families with complex regulation, their regulation is difficult to interpret. Not surprisingly, phytohormone levels are also abnormal in root-knot nematode–infected roots, providing an additional level of complexity in understanding plant responses to nematode infection (Glazer et al., 1986).

Changes in gene expression suggestive of a stress or defense response have been observed after infection with either cyst or root-knot nematodes. For example, changes in gene expression in potato leaves after root infection by the cyst nematode *G. rostochiensis* include the induction of pathogenesis-related proteins (Hammond-Kosack et al., 1989). Root-knot nematode juveniles, which cause considerably less cell damage when they invade than do cyst nematodes, also induce apparent defense genes, although there are differences in the pattern and timing of induction between the two groups of nematodes.

In tomato roots infected with root-knot nematodes, genes with homology to several known plant defense genes (including peroxidase, chitinase, lipoxygenase, and proteinase inhibitors) are induced locally within 12 hr of inoculation (Lambert, 1995; B. Ferrie and V.M. Williamson, unpublished data). A gene encoding a catalase, which is induced after infection with root bacteria, is induced both locally and systemically in potato after infection with *Meloidogyne incognita* (root knot) as well as *G. pallida* (cyst) (Niebel et al., 1995). Hansen et al. (1996) found that the wound-inducible *wun1* promoter directed expression of β-glucuronidase (*GUS*) that was rapidly and transiently induced by cyst nematodes in potato but only produced a modest and much slower induction after root-knot nematode infection. A similar pattern of expression was seen with the *gst1* gene promoter in potato (Strittmatter et al., 1996). The tomato gene *hmg2*, which encodes an HMG-CoA reductase that is induced by other pathogens, is upregulated after root-knot nematode infection (Cramer et al., 1993). mRNA levels of extensin, a family of genes encoding glycoproteins that form a major component of plant cell walls and are induced in plant defense responses, are significantly increased in *M. javanica*–induced galls at 1 week after infection (Niebel et al., 1993) as well as in tomato root tips by 12 hr after infection (Lambert, 1995). Strong induction of extensin is ob-

served in root-knot nematode infection, but weak and transient expression is observed after cyst nematode infection. Because cyst nematodes cause much more wounding than do root-knot nematodes, extensin induction in the latter group may be due to factors other than wounding. For example, extensin may play a role in the nematode-induced alterations in feeding site or gall development. The callose deposition around the stylet tip in a parasitized cell may also reflect a wound response, because plant cells commonly respond to mechanical injury or fungal infection by rapid deposition of callose along the inner surface of the affected wall (Aist, 1976).

Genes that may be important in development and maintenance of feeding sites have been investigated as a way to understand better the establishment of these structures by the nematode. As described above, early events in the development of giant cells include the proliferation of nuclei and concomitant endoreduplication of DNA, indicating that regulation of progression through the cell cycle is altered. Transcriptional activation of the cell cycle markers *cdc2a*, which encodes a cyclin-dependent kinase, and *cyc1At*, which encodes a mitotic cyclin, is observed in Arabidopsis roots after infection by root-knot nematodes (Niebel et al., 1996). Transcriptional activation of these markers is not observed during the first few days after infection, while the nematode migrates through the root, but occurs within hours of giant cell initiation. A similar induction pattern for both of these genes is seen near and inside the syncytium after infection with the cyst nematode *H. schachtii*, even though syncytial cells do not undergo the same rapid proliferation of nuclei that is seen in giant cells. However, incorporation of tritiated thymidine in developing syncytia indicates that DNA endoreduplication does occur in these cells (Endo, 1971).

Studies on expression of the reporter gene *GUS* fused to the promoter of *TobRB7*, a tobacco gene normally expressed in root tips, showed that this promoter also directs expression in giant cells (Opperman et al., 1994a). Molecular dissection of the *TobRB7* promoter revealed that the 300 bp of DNA immediately upstream from the transcript start direct *GUS* expression in giant cells but not in root tips. Interestingly, this control region does not direct *GUS* expression in cyst nematode–induced syncytia. *TobRB7* encodes a membrane protein believed to function as a water channel. Antisense constructs of this gene suppressed nematode reproduction, suggesting that expression is required for successful feeding site development or maintenance (Opperman et al., 1994c).

Searches for additional plant genes with increased expression in feeding sites have been hampered by the very small size of the feeding sites. Nevertheless, cDNA libraries have been constructed from RNA of isolated giant cells, galls, and syncytia, and genes that are upregulated after nematode infection have been identified from these libraries (Gurr et al., 1991; Wilson et al., 1994; Van der Eycken et al., 1996). A giant cell cDNA library constructed by a subtractive approach included sequences encoding a plasmalemmal proton ATPase, a putative Myb-type transcription factor, and the largest subunit of RNA polymerase II (Bird and Wilson, 1994). Among genes identified after differential screening of a cDNA library from galled roots were those encoding extensin genes and one with homology to the cotton gene *Lea14-A*, which encodes a late embryogenesis-abundant protein (Van der Eycken et al., 1996).

In a complementary approach, transformation of plants with "promoter traps" by random integration of promoterless *GUS* constructs has identified regions of the genome in which expression of *GUS* is increased or decreased upon nematode infection (Goddijn et al., 1993; Gheysen et al., 1996). Transgenic plants with a variety of tissue specificities and differences in timing of *GUS* expression have been identified in Arabidopsis. These will be valuable tools for dissecting the sequence and timing of events involved in the host response.

It is not just the genes whose expression increases that are of interest. Many genes are downregulated in giant cells or developing galls as well as systemically in the plant after nematode infection (Hammond-Kosack et al., 1989; Goddijn et al., 1993). The powerful cauliflower mosaic virus 35S promoter has been reported to be silenced in developing galls within days after root-knot nematode infection (Goddijn et al., 1993). It may be that host genes are actively turned off by the nematode to repress defense responses. Alternatively, they may be turned off due to a more general response to nematode invasion.

The changes in gene expression identified so far are likely to be several steps downstream from the initial plant responses to signals from the nematode. Strategies aimed at identifying the primary events in the response to nematode signals include the identification of early changes in gene expression after infection (Williamson et al., 1994) and the analysis of promoter elements and putative transcription factors that control nematode-induced genes (C. Fenoll, personal communication). Characterization of nematode secretions may also identify molecular triggers whether these secretions are injected directly into the cytoplasm or are deposited outside the plasma membrane (Figure 3). In either case, specific compounds in the secretions or products produced as a result of these secretions could bind to plant cell receptors to elicit a signal transduction cascade to modulate gene expression. Alternatively, they may enter the nucleus and directly modify gene expression in the recipient cell.

HOST PLANT RESISTANCE

Plants are defined as resistant to nematodes when they have reduced levels of reproduction (Trudgill, 1991). Nematode resistance genes are present in several crop species and are an important component in many breeding programs, including those for tomato, potato, soybean, and cereals (Roberts, 1992). Resistance can be broad, that is, effective against several nematode species, or narrow, that is, controlling only specific biotypes of a species (also variously referred to as races or pathotypes). Several dominant or semidominant resistance genes have been identified and mapped to chromosomal

Table 1. Mapped Nematode Resistance Loci[a]

Crop	Species of Origin	Locus	Nematode	Genetic Location	Reference
Tomato	*L. peruvianum*	*Mi*	*M. incognita* *M. javanica* *M. arenaria*	Chromosome 6	Messeguer et al. (1991) Ho et al. (1992)
Tomato	*L. peruvianum*	*Mi3*	*M. incognita* *M. javanica*	Chromosome 12	Yaghoobi et al. (1995)
Tomato	*L. pimpinellifolium*	*Hero*	*G. rostochiensis*	Chromosome 4	Ganal et al. (1995)
Potato	*Solanum tuberosum* spp *andigena*	*H1*	*G. rostochiensis*, pathotypes *Ro1* and *Ro4*	Chromosome 5	Pineda et al. (1993) Gebhardt et al. (1993)
Potato	*S. spegazzinii*	*Gro1*	*G. rostochiensis*, pathotypes *Ro1* and *Ro5*	Chromosome 7	Ballvora et al. (1995)
Potato	*S. spegazzinii*	*Gpa*	*G. pallida*, pathotypes *Pa2* and *Pa3*	Chromosome 5	Kreike et al. (1994)
Potato	*S. vernei*	*GroV1*	*G. rostochiensis*, pathotype *Ro1*	Chromosome 5	Jacobs et al. (1996)
Potato	*S. bulbocastanum*	R_{Mc1}	*M. chitwoodi*	Chromosome 11	Brown et al. (1996)
Sugar beet	*B. patellaris*	$Hs1^{pat-1}$	*H. schachtii*	Chromosome 1	Salentijn (1992)
Soybean	*Glycine max*	Rhg_4	*H. glycines*, race 3	Linkage group A	Webb et al. (1995)
Wheat	*Triticum aestivum*	*Cre*	*H. avenae*	Long arm of chromosome 2B	Williams et al. (1994)
Wheat	*T. tauschii*	*Cre3*	*H. avenae*	Long arm of chromosome 2D	Eastwood et al. (1994)

[a] Representative list of mapped dominant nematode resistance loci.

locations or linkage groups (Table 1). For other sources of resistance, inheritance is polygenic or in some cases recessive (Trudgill, 1991; Kreike et al., 1993; Faghihi et al., 1995; Wang and Goldman, 1996). In some cases, polygenic resistance has been resolved into major genes, which are genetically dominant, and minor genes, which may modulate the response (Kreike et al., 1994; Webb et al., 1995). With many of these resistance genes, a localized necrosis or hypersensitive response, resembling that described for other pathogen resistance genes (see Dangl et al., 1996, in this issue; Hammond-Kosack and Jones, 1996, in this issue), is associated with nematode infection.

One of the best characterized nematode resistance genes is *Mi*, which confers resistance to several root-knot nematode species in tomato. *Mi*-mediated resistance is characterized by a localized necrosis of host cells near the invading nematode (Dropkin, 1969). The earliest visible indications of the hypersensitive response occur at ~12 hr after inoculation of roots with nematode juveniles (Paulson and Webster, 1972). This timing suggests that attempts to initiate a giant cell by the nematode elicit the response. Interestingly, resistance mediated by *Mi* is lost at elevated temperatures (Dropkin, 1969). Temperature shift experiments showed that determination of resistance occurs during the first 24 to 48 hr after infection and that once this time period is passed, resistance is not triggered even at the permissive temperature (Dropkin, 1969).

For other resistance genes, the host response appears to occur with different timing and tissue localization than it does for *Mi*. For example, *H1*-mediated resistance of potato to *G. rostochiensis* is characterized by necrosis of tissue around the invading nematode. However, despite the initial necrosis, the feeding site begins to develop and the nematode becomes sedentary (Rice et al., 1987). In time, however, the feeding site becomes surrounded by necrosing tissues and eventually collapses. The few nematodes that do develop on *H1* potato plants are mostly male, a sign of poor nutrition for the nematode.

Whether the localized cell death is directly responsible for the resistance or is a secondary response is not known. Additional molecular changes occur rapidly after infection of resistant plants. For example, activity levels of the enzymes phenylalanine ammonia-lyase and anionic peroxidase, which are induced early in the resistance response to many other pathogens, also increase in resistant tomato after nematode inoculation (Brueske, 1980; Zacheo et al., 1993). Furthermore,

differential screening of a cDNA library from root tips infected for 12 hr with root-knot nematodes led to the identification of several defense gene homologs. However, most of these are also induced in susceptible plants, although there are differences in the extent and timing of induction (Williamson et al., 1994; Lambert, 1995; B. Ferrie and V.M. Williamson, unpublished data). Consequently, the role, if any, of the induced genes in nematode resistance has yet to be determined.

Intensive efforts to clone nematode resistance genes are currently in progress (Ho et al., 1992; Klein-Lankhorst et al., 1994; Ballvora et al., 1995; Ganal et al., 1995), but so far none have been positively identified. It will be interesting to see whether nematode resistance genes resemble the already cloned pathogen resistance genes in sequence and structure (Staskawicz et al., 1995; Bent, 1996, in this issue). A few lines of evidence suggest that they will. First, nematode resistance genes are frequently clustered with other pathogen resistance genes in plant genomes. For example, in tomato, *Mi* maps within 1 centimorgan of the fungal resistance genes *Cf2* and *Cf5* (Dickinson et al., 1993; Dixon et al., 1996) and even closer to *Meu1*, an aphid resistance gene (Kaloshian et al., 1995). Also, the potato nematode resistance gene *Gpa* is tightly linked to the viral resistance gene *Rx2* and a fungal resistance gene, *R1* (Kreike et al., 1994). Second, homologs of cloned pathogen resistance genes have been identified that map physically very near to *Mi* (within 60 kb; J. Bodeau and V.M. Williamson, unpublished data), *Grol* (Leister et al., 1996), and *Cre3* (E. Lagudah, O. Moullet, and R. Appels, personal communication). In all three cases, these resistance gene homologs contain the nucleotide binding domain and leucine-rich repeat motifs found in several cloned resistance genes.

GENETICS OF VIRULENCE IN NEMATODES

Nematodes in the Heteroderidae have diverse means of reproduction. Many cyst nematodes reproduce exclusively by sexual mating, whereas many of the most damaging root-knot nematode species (including *M. incognita*, *M. javanica*, and *M. arenaria*) reproduce by mitotic parthenogenesis, a mechanism generating clonal progeny in which eggs are produced by mitotic, not meiotic, divisions. Other root-knot nematode species, including the important pathogen *M. hapla*, reproduce by facultative meiotic parthenogenesis, in which both parthenogenic and sexual reproduction can occur (Triantaphyllou, 1985).

Intraspecific genetic variation in host range and response to specific resistance genes is high, especially for the sexually reproducing plant parasitic nematode species. This heterogeneity has made it difficult to breed for nematode resistance in crops such as soybean and potato (Bakker et al., 1993; Faghihi et al., 1995). However, inbred populations of *G. rostochiensis* have been developed, and controlled genetic crosses between individuals from these populations have been performed. These genetic studies demonstrated that a gene-for-gene relationship between the *H1* resistance gene in potato and a dominant avirulence gene in the pathogen controls this interaction (Janssen et al., 1991). Inbred lines of *H. glycines* have also been produced, and progress has been made toward developing a genetic map for this species (Opperman et al., 1994b). This map will provide a valuable resource for genetic investigation of virulence and other aspects of host–pathogen interactions.

Parthenogenic root-knot nematode species have lower but still considerable variability within and between species for host range and virulence/avirulence (Roberts, 1995). A well-characterized example is the existence of populations of root-knot nematodes that are virulent on plants carrying *Mi*. Such populations have been identified both in unselected isolates and after selection on resistant plants in the greenhouse (Bost and Triantaphyllou, 1982; Jarquin-Barberena et al., 1991). Greenhouse studies on the development of virulence to *Mi* showed a progressive increase in virulence after prolonged selection on resistant plants, suggesting that several genes are involved (Triantaphyllou, 1987; Castagnone-Sereno et al., 1994). On the other hand, comparison of soluble proteins from closely related strains of virulent and avirulent adult nematode females on 2-D polyacrylamide gels showed one additional protein spot in the avirulent female (Dalmasso et al., 1991). The gene encoding this protein is a candidate for a root-knot nematode avirulence gene. Unfortunately, due to the lack of sexual reproduction, the inheritance of avirulence and virulence cannot be directly tested but must await development of new strategies and techniques, including, perhaps, transformation of phytopathogenic nematodes.

PROSPECTS FOR ENGINEERED RESISTANCE

Although the incorporation of natural resistance is a major component of current nematode management in these days of reduced pesticide use, there are many crops for which appropriate resistance loci are not available. As nematode resistance genes are cloned, it may be possible to transfer them to additional hosts; however, it is not certain that genes will function effectively in heterologous hosts. Furthermore, acquisition of virulence by nematodes may shorten the effective utility of this approach. To fill the gap, a variety of strategies to engineer synthetic resistance are being developed (reviewed in Sijmons et al., 1994; Gheysen et al., 1996).

One objective is to engineer plants to express genes that are detrimental to the nematode. Potential anti-nematode genes include those encoding proteinase inhibitors, collagenase (the nematode cuticle is composed largely of collagen), or toxins. A second approach is to transform plants with genes encoding monoclonal antibodies or single chain antibodies (plantibodies) to specific stylet secretions or other components of the nematode in an attempt to block the establishment of a feeding site (Baum et al., 1996; Rosso et al., 1996). Another

promising strategy is to transform the plant with constructs designed to interrupt feeding cell development. Disrupting the development of the feeding site is a particularly attractive approach because nematodes are totally dependent on the feeding site for their nourishment. Feeding site disruption could be accomplished either by specifically expressing a phytotoxin in feeding cells or by inhibiting processes required for feeding cell development. As this type of resistance focuses on plant processes rather than on those of the nematode, it is likely to be difficult for the nematode to evolve to circumvent the resistance. An important caveat for this strategy is that the promoter must be very specific so as not to allow the toxin to destroy cells that are essential for plant development and reproduction. An alternative approach is to incorporate a second component, a neutralizing gene that is constitutively expressed in the plant except in feeding structures (Sijmons et al., 1994).

For most of these strategies, it is too early to tell whether they will be effective. Early signs of success have been obtained with the expression of specific proteinase inhibitors in tomato root cultures; in this case, a detrimental effect was seen on growth and development of *G. pallida* (Urwin et al., 1995). Transgenic plants expressing an antisense construct of *TobRB7* under a giant cell–specific promoter also had a strong detrimental effect on nematode survival in greenhouse and field trials, with galling reduced by ~70% compared with that in controls (Opperman et al., 1994c).

MODEL SYSTEMS

Model organisms have been instrumental for advancing current knowledge in many fields of biology. Two models that have potential for providing insights into plant–nematode interactions are the plant *Arabidopsis thaliana* and the animal *Caenorhabditis elegans*.

Arabidopsis is a host for several species of root-knot and cyst nematodes (Sijmons et al., 1991). The thin, transparent roots have permitted video microscopy studies that have greatly enhanced our understanding of nematode behavior inside roots. Furthermore, a large array of transgenic Arabidopsis lines containing reporter gene constructs whose expression is modulated by nematode infection has already been generated (Goddijn et al., 1993; Gheysen et al., 1996). Analysis of these lines should produce information on the timing and sequence of events during the plant's response to nematode infection. It should also be possible to screen directly for Arabidopsis mutants with altered responses to nematode infection. Once the corresponding genes are mapped, positional cloning should be relatively straightforward. Unfortunately, no resistance to nematodes has yet been found in Arabidopsis, although there seem to be differences in infectivity between ecotypes (Sijmons et al., 1991). Only a limited number of nematode species have been tested, however, and it may be that nematode resistance will be identified after additional screening with inbred nematode lines or additional species.

The massive body of work on the free-living nematode *C. elegans* is likely to have applications for plant pathogenic nematode studies (Riddle and Georgi, 1990; Hodgkin et al., 1995). Many molecular and developmental peculiarities of the phylum are conserved between *C. elegans* and plant parasites. For example, plant parasitic nematodes contain trans-spliced mRNAs with leader sequences similar or identical to those found in *C. elegans* (Ray et al., 1994; Stratford and Schields, 1994). Molecular tools developed for *C. elegans*, such as procedures for transformation, in situ hybridization, and transposon-mediated gene disruption, will surely contribute to investigation of an important and fascinating group of organisms, the plant parasitic nematodes.

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