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Engineering Natural and Synthetic Resistance for Nematode Management[1]

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Abstract: Bioengineering strategies are being developed that will provide specific and durable resistance against plant-parasitic nematodes in crops. The strategies come under three categories: (i) transfer of natural resistance genes from plants that have them to plants that do not, to mobilize the defense mechanisms in susceptible crops; (ii) interference with the biochemical signals that nematodes exchange with plants during parasitic interactions, especially those resulting in the formation of specialized feeding sites for the sedentary endoparasites—many nematode genes and many plant genes are potential targets for manipulation; and (iii) expression in plant cells of proteins toxic to nematodes.

Key words: cystatins, enzyme inhibitors, giant cells, hypersensitivity, lectins, natural resistance genes, nematodes, nematode genes, nematode-induced promoters, plant defense, plant genes, syncitia, synthetic resistance, toxins, transformation, transgenic plants.

Effective resistance against plant-parasitic nematodes is uncommon in many crops. Yet crop resistance is the most environmentally friendly and cost-effective means of reducing yield losses in agriculture. Hence, considerable emphasis is placed on molecular-assisted breeding of natural resistance genes and engineering of synthetic resistance genes (Jung et al., 1998). The use of molecular markers tagging natural resistance genes hastens the development of resistant cultivars. Molecular markers also have become essential tools to map and clone natural resistance genes, so that they can be introduced in virtually any crop using transgenic techniques. The development of synthetic resistance derives from our detailed understanding of plant and nematode physiology and of the molecular signals exchanged (Gheysen, 1998; Williamson and Hussey, 1996). The signalling molecules are often proteins, produced by plants or by nematodes. Plant genes encoding these proteins can be manipulated and transferred back into the plants, with the expectation that plants expressing disabled signal proteins will not sustain parasitic infection since the blocked molecular exchange with the parasite commonly results in cell death (apoptosis). Nematode signal proteins can also be identified, and it is hoped they will lead to the identification of plant proteins with which they interact. Synthetic resistance can also be derived from the expression of toxic molecules in the plant, either to affect nematodes directly or to interfere with the formation and maintenance of their feeding cells. While this review is comprehensive, its objective is to reach the majority of nematologists whose expertise is outside of molecular biology. For this reason I have included a brief introduction to *Agrobacterium* mediated transgenesis and some definition of terms. While I have written in a generally enthusiastic manner, it is important to note that the recent field trials of transgenic crops expressing synthetic resistance against nematodes have been disappointing. A better understanding of plant-nematode interactions is still required before nematologists can contribute effective and widespread transgenic nematode resistance to agricultural crops.

The Tools of Genetic Transformation

In many cases a natural transgenesis mechanism evolved by the plant-pathogenic bacterium *Agrobacterium tumefaciens* is used (Gheysen et al., 1992). Plants react to wounding by secreting toxic molecules to keep pathogens away while cell barriers are being repaired. *Agrobacterium tumefaciens* is not only immune to these toxic molecules

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but also is attracted to some of them, especially a phenol called acetosyringone. When this flagellate bacterium is in the rhizosphere and root tissue is wounded, it swims toward the wounded cells, attracted by acetosyringone and other phenols. The virulence genes of this bacterium are positioned on a large plasmid called the tumor-inducing plasmid (Ti). There are several genes on the Ti plasmid that are in a special domain called transfer DNA (T-DNA); these are the genes that will be transferred into the plant cell nucleus. En route toward the wounded plant cells, the bacterium is synthesizing virulence proteins. When it makes contact with the plasma membrane of a wounded cell, the bacterium makes copies of its T-DNA and the virulence proteins transport one or several copies across the bacterial plasma membrane, across the plant cell plasma membrane, and into the nucleus. Once inside the nucleus of the plant cell, the bacterial T-DNA is integrated at random into a plant chromosome. The T-DNA genes travel with their own controlling sequences and are immediately expressed in the plant cell. The bacterial proteins produced by the plant redirect much of the cell processes to make the special carbohydrates that the bacteria require. The transgenesis mechanism of *A. tumefaciens* is commonly used to engineer plants by replacing the genes and control sequences of the T-DNA with other genetic constructs.

One limitation of this technique is that few monocotyledonous plants are susceptible to *A. tumefaciens* infection. Biolistics is another method that circumvents this limitation. By shooting through a "gene gun," pellets coated with DNA fragments containing gene constructs are introduced into the nuclei of epidermis cells or deeper tissues. This technique is notably inefficient as it is a rare DNA fragment that is integrated into a plant chromosome, but large numbers of biolistic experiments can be performed in a short time. The next stage, with either method, is to regenerate a whole plant from one genetically transformed cell. This is a complex level of tissue culture with antibiotics to eliminate *Agrobacterium,* selection agents (mostly herbicides and antibiotics) to prevent untransformed cells from multiplying, and regular plant hormones. As with all genetic engineering approaches, the transfer of natural or synthetic resistance genes to previously susceptible host plants can use either transgenesis protocol.

Most plants have evolved biochemical defense processes that most nematodes are not able to breach, i.e. most plants are immune to most nematodes—they are non-hosts. Therefore, these plants constitute a potential reservoir of natural resistance genes, and since these genes can be cloned and transferred among plant species there is a virtually unlimited pool of genes to develop effective and durable resistance against nematodes.

Natural Resistance Genes

The major genes for resistance already known to breeders are prime candidates for engineering (Williamson, 1998). However, since the number of effector genes that can be transferred into a plant is currently limited to two or three, only major resistance genes are considered. To transfer a natural resistance gene by genetic engineering, it must first be localized (mapped) on the plant chromosome and its sequence must be determined. A basic strategy for localizing plant resistance genes is map-based, i.e. DNA fragments (markers) associated to (surrounding) the resistance gene are found. These fragments can be RFLP (Restriction Fragment Length Polymorphisms) markers, RAPDs (Randomly Amplified Polymorphic DNA), AFLPs (Amplified Fragment Length Polymorphisms), or SSRs (Simple Sequence Repeats) that are associated with a fragment of DNA that always cosegregates with the resistance trait in genetic crosses. The next step is to make a cDNA library (a collection of small DNA fragments where all genes are represented) and identify where the markers are on various clones (DNA fragments). The more markers that are identified and the closer some of them are to the gene, the shorter the fragment of chromosome that must be sequenced. To

that effect, several techniques have been developed to increase the density of markers near the gene of interest: (i) bulked segregant analysis (Hallden et al., 1997; Yaghoobi et al., 1995); (ii) comparative genome mapping, which uses mapping information from one species to hypothesize linkage relationships in other species; (iii) integrated mapping, which integrates linkage maps from independent populations (Concibido et al., 1996).

Another strategy to isolate a gene is "transposon tagging." Transposable elements are short DNA sequences that move around the genome of plants spontaneously, causing mutations when they insert themselves into a gene. Transposons are treated as tags to locate a particular gene when its function is altered by transposon insertion (Abad, 1994).

The predicted products of more than a dozen natural plant resistance genes against fungal, bacterial, and viral pathogens have been characterized. They are all proteins, and most of them have striking structural similarities, suggesting that resistance mechanisms are conserved. In other words, a family of related genes in the plant is devoted to the activation of defenses against pests and pathogens (Baker et al., 1997; Bent, 1996; Gebhardt, 1997; Hammond-Kossack and Jones, 1997; Jones and Jones, 1996; Parker and Coleman, 1997). The resistance gene products are usually involved with the signal that triggers the transduction cascade leading to a hypersensitive response (HR), a form of programmed cell death (apoptosis) of the parasitized cells. Moreover, there may be many natural resistance genes against nematodes involved with detecting the presence of the parasites and triggering a hypersensitive reaction. Two natural resistance genes against cyst and root-knot nematodes have been cloned from sugarbeet and tomato, and the sequence of their proteins suggests that their role is to detect chemical signals from the nematodes (Cai et al., 1997; Milligan et al., 1998).

The gene from sugarbeet was isolated from a related wild species, *Beta procumbens.* Wild beets are not closely related to cultivated species and their chromosomes do not pair properly, so recombination mapping, the usual approach to locate a resistance gene, could not be used. Instead, a short DNA sequence that always hybridized with the DNA of wild beets and the resistant hybrids of cultivated sugarbeets was used to map the resistance gene. A collection of hybrid beets contained many plants carrying the resistance gene on small chromosomal translocation segments originally from wild beets. The DNA sequence was used as a probe to identify resistant plants, and especially the resistant plant with the smallest chromosome segment from the wild species. The nematode resistance gene was finally obtained from a cDNA library through cross-hybridization with a yeast artificial chromosome library (a collection of all the beet genes held in yeast cells) of that resistant plant. The cloned gene, $Hs1^{pro\text{-}1}$, was transferred to susceptible sugarbeet and conferred resistance against the sugarbeet cyst nematode (Cai et al., 1997; Kleine et al., 1998). A second sugarbeet cyst nematode resistance gene has also been found at the same locus (Sandal et al., 1997).

The other nematode resistance gene recently isolated is *Mi* from tomato, *Lycopersicon esculentum* (Milligan et al., 1998). The *Mi* gene, used since the 1940s when it was introgressed from its wild relative *L. peruvianum,* is important because it confers resistance to four of the major root-knot nematode species (Brown et al., 1997; Williamson et al., 1994a) and can be used to study the biochemistry of susceptible and resistant plants. Many years of intense efforts were required to localize this gene because of a lack of recombination in the region of the gene in tomato plants that have received the introgressed DNA of the wild species, *L. peruvianum,* through standard breeding (Ho et al., 1992; Liharska et al., 1996; Messeguer et al, 1991; Williamson et al., 1994b). Thus, large numbers of tomato plants had to be used in breeding experiments to screen for recombinants and to identify recombinants in *L. peruvianum* and *L. esculentum* populations (Kaloshian et al., 1998). Data from re-

combinant analyses circumscribed *Mi* to a 65-kb fragment of DNA, of which 52 kb were sequenced. Two genes, *Mi*-1.1 and *Mi*-1.2, were found, but only *Mi*-1.2 conferred resistance to a previously root-knot nematode susceptible tomato cultivar (Milligan et al., 1998). The *Mi*-1.2 gene is closely related to another tomato gene for resistance against *Pseudomonas syringae*. The *Mi*-1.2 protein has structural motifs found in the family of proteins that protect plants from viral, bacterial, and fungal pathogens. The *Mi* gene confers resistance in tomato against aphids (Rossi et al., 1998). This dual resistance suggests some shared parasitic interactions of aphids and root-knot nematodes with their host plants. Singularly, aphids, potato cyst nematodes, and root-lesion nematodes are also susceptible to the same lectin (Boulter et al., 1990; Burrows et al., 1998).

A family of resistance genes, *Cre,* against the cereal cyst nematode has been identified and mapped in wheat (Lagudah et al., 1997). The conserved domains of these resistance genes were used to identify homologous resistance genes from other cereal plants. Seah et al. (1998) isolated several genes from barley with a high degree of homology with *Cre3,* but it is too early to speculate whether these sequences function effectively as resistance genes in barley or other cereals. This strategy also was used to find genes with homology to *Gro1* (a gene conferring resistance to *Globodera rostochiensis*) in potato (Leister et al., 1997). Other projects that use a map-based cloning strategy to localize resistance genes are at the marker identification stage. They include genes for resistance against both species of potato cyst nematodes: *H1 and Gpa* in potato, and *Hero* in tomato (Ballvora et al., 1995; Bradshaw et al., 1998; Ganal et al., 1995; Gebhardt et al., 1993; Jacobs et al., 1996; Leister et al., 1997; Niewohner et al., 1995; Pineda et al., 1993; Rouppe Van der Voort et al., 1997, 1998, 1999); *Ha2* and other genes in barley against the cereal cyst nematode (Barr et al., 1998; Kretschmer et al., 1997; Taylor et al., 1998; Williams et al., 1994); several genes against various races of the soybean cyst nematode (Chang et al., 1997; Concibido et al., 1997; Danesh et al., 1998; Diers et al., 1997; Heer et al., 1998; Li et al., 1996; Mahalingam and Skorupska, 1995; Matthews et al., 1998; Mudge et al., 1997; Vierling et al., 1996); and several genes against root-knot nematodes in tobacco (Yi et al., 1998), tomato (Yaghoobi et al., 1995), soybean (Tamulonis et al., 1997a, 1997b), sweet potato (Ukoskit et al., 1997), potato (Brown et al., 1996), peanut (Burow et al. 1996; Garcia et al., 1996), and peach rootstocks (Lu et al., 1998).

While the putative function of natural plant resistance genes can be known only after they are isolated, other plant genes that are specifically activated in nematode feeding cells can be determined. The expectation is that once these nematode activated genes are identified, synthetic resistance genes can be designed to disable their function (antisense strategies) or their promoters or transcription factors, and abort the formation of feeding cells. For a recent review, see Fenoll et al. (1997).

Nematode Feeding Cell-Specific Genes

Many genes expressed in nematode feeding cells, or the regulatory regions that control these genes, have been isolated (Barthels et al., 1997; Bird, 1996, 1998; Bird and Wilson, 1994; Hermsmeier et al., 1998; Niebel et al., 1993, 1995, 1996; Van der Eycken et al., 1996; Wilson et al., 1994). Wilson et al. (1994) cloned cDNAs from 150 mature giant cells dissected from tomato roots infected with root-knot nematode. Starting with a cDNA library of 2 million DNA fragments from infected roots, after a rigorous subtraction against cDNA of uninfected roots, 220 cDNAs remained after the subtraction steps (Bird and Wilson, 1994). The analysis of this gene library is providing clues about the kind of plant genes that are expressed in giant cells. Most of these genes are expressed in other tissues of the plant at other times, and many are "pioneers," i.e. not related to other genes previously isolated from plants or other organisms (Bird, 1996, 1998).

A promoter-tagging technique is used to

isolate feeding cell-specific genes and their promoters (the promoter is the DNA domain that the RNA polymerase needs to bind to before it can start reading the DNA strand—i.e. the control switch) (Barthels et al., 1997; Fenoll et al., 1997). The promoters of these genes are isolated by transforming plants to express a reporter gene in a promoterless construct. When the transgenic plants are infected with cyst or root-knot nematodes, reporter gene expression in syncytia or giant cells means that the construct has integrated behind the promoter of a gene that is active in these feeding cells. The unknown promoter and gene are then isolated using inverse PCR.

A Promoter Induced by Root-Knot Nematodes

A dissection of the promoter of a tobacco aquaporin gene (*tobRB7*) has serendipitously yielded a control sequence specifically triggered by root-knot nematodes. Opperman et al. (1994) made a deletion series at the 5′ end of the tobacco RB7 promoter and used the truncated promoters to drive GUS expression (β-glucuronidase, a commonly used reporter gene) in tobacco. When transgenic tobacco plants were infected with root-knot nematodes, those plants transformed with the shortest promoter sequence (300 bp) showed GUS expression only in the giant cells and nowhere else in the roots. This truncated promoter functioned as a nematode-specific trigger, so it was subsequently used to direct the expression of a toxic protein (barnase, a protein that digests RNA) to abort the formation of giant cells in tobacco. Although the plants were resistant and nematodes did not develop, the truncated promoter was not exclusively giant cell-specific, as there was some expression of the toxic protein in other organs, resulting in severe growth alterations in the plants. Cotton was transformed to express an attenuated barnase to abort the formation of giant cells, or independently transformed with a full-length cDNA antisense construct of the cotton homolog of *tobRB7* to interfere with the normal function of giant cells (Robinson et al., 1998). Plants expressing the attenuated barnase were not resistant. Plants expressing the antisense had a low level of resistance to *Meloidogyne incognita* but no resistance to *Rotylenchulus reniformis*.

Other Promoters Used to Interfere with Feeding Cell Formation

The cauliflower mosaic virus 35S promoter, the nopaline synthase promoter from *A. tumefaciens,* and several other strong constitutive promoters are highly active in roots. The 35S promoter was first thought to be silenced or seriously down-regulated in nematode feeding cells, as shown in tobacco and in *Arabidopsis*. However, the use of a more sensitive marker (GFP, green fluorescent protein) and a dissection of its six domains showed that it is not down-regulated in nematode feeding cells, where it delivers effective levels of gene expression (Bertioli et al., 1999; Goddijn et al., 1993; Goverse et al., 1998; Urwin et al., 1997b).

In tomato plants, the promoter of a defense-related gene (hydroxymethylglutaryl CoA reductase), triggered by fungal and bacterial pathogens, drives strong GUS expression in root-knot nematode giant cells (Cramer et al., 1993). However, even though this "defense gene" is expressed, it does not prevent the nematodes from establishing and maintaining functional feeding sites. The promoter of a hemoglobin gene from a nitrogen-fixing, non-leguminous plant exhibits a complex pattern of control that could be utilized (Ehsanpour and Jones, 1996). When root-knot nematodes develop in roots of transgenic tobacco expressing GUS under the control of this hemoglobin gene promoter, there is little GUS expression in the giant cells during the first 2 weeks after giant cell initiation, in contrast to the high level of expression in other root tissues. However, GUS expression is high in the giant cells at later stages of infection, 3 to 6 weeks after giant cell initiation, and when females start producing eggs. Expression in other tissues declines drastically.

A wound-inducible promoter, *wun1* from

potato, is strongly up-regulated when cyst nematodes enter potato roots but not when root-knot nematodes enter the same roots (Hansen et al., 1996). This matches what we know of the behavior of these nematodes during infection; the intracellular migration of cyst nematode juveniles damages cortical tissue much more than the intercellular migration of root-knot juveniles. Another wound-inducible promoter from asparagus was used in transgenic tobacco roots to control GUS expression only in traumatized cells (Warner et al., 1993). GUS expression is evident only in root hairs and tissues where *Pratylenchus penetrans* feeds and where *Meloidogyne hapla* is established (unpublished).

Nematode Virulence and Parasitism Genes

Parasitism results from the exchange of biochemical signals between nematodes and plants. Identifying nematode signal products required for the interaction provides other ways to interfere with the signals and abort the formation of feeding cells. The current paradigm for cyst nematodes, as well as for root-knot nematodes, is that syncytial or giant cells are formed when the juvenile nematodes release esophageal gland secretions through their stylet into plant tissue. Not everybody ascribes to this view; other nematode secretions or surface molecules could be involved (Jones and Robertson, 1997). In any case, nematode genes that produce proteins secreted through the stylet provide potential targets to interfere with the nematode-plant interaction. Esophageal secretions contain proteins, and one or several of these proteins may function like plant transcription factors (proteins that trigger or interfere with the expression of a gene), playing a role in plant cell gene deregulation (Gheysen, 1998; Williamson and Hussey, 1996). Obviously, it takes many plant genes to turn regular plant cells into hypertrophied, multinucleated cells dedicated to nematode feeding. If it is a nematode product that is indeed at the origin of this transformation, once identified, this signal molecule can be a target for interference.

Several groups in Europe and the United States are collaborating to identify esophageal gland proteins from root-knot and cyst nematodes with monoclonal antibodies (Mabs). The nematodes release esophageal secretions through their stylet when immersed in solutions of neurotransmitters. The secretions are collected and injected into the spleen of a mouse to make Mabs. These antibodies are then screened to identify those that bind to a nematode secretory protein, or to a protein in the esophageal glands. Recently, cellulases and cellulase binding proteins were recognized in the esophageal glands of *G. rostochiensis, H. glycines,* and *M. incognita,* using Mabs and RNA fingerprinting, and the secretion of *H. glycines* cellulases was demonstrated in planta (Ding et al., 1998; Rosso et al., 1999; Smant et al., 1998; Wang et al., 1999; Yan et al., 1998). Other Mabs have identified additional nematode secretory proteins whose functions have yet to be characterized (Davis et al., 1994; De Boer et al., 1996; Goverse et al., 1994). The coding regions of the variable domain of several mouse antibody genes have been cloned and expressed in plants (plantibodies) as monoclonal antibodies or single-chain antibodies (Baum et al., 1996; Rosso et al., 1996; Schots et al., 1992; Stiekema et al., 1997). The expectation is that expression of these plantibodies in a transgenic plant will abort the formation of nematode feeding cells by binding to and altering the function of key proteins of the nematode esophageal secretions.

As with other pests and pathogens, specific gene products must be involved in the specificity parasitic nematodes display toward particular plants. When these genes are identified and cloned, new opportunities will arise to interfere with the host-parasite interactions. A genetic analysis of soybean cyst nematode parasitism on resistant and susceptible soybeans has been started with highly inbred (homozygous) lines of the cyst nematode (Dong and Opperman, 1997; Dong et al., 1997). Several nematode genes control reproductive ability

on resistant soybean cultivars, and RAPD and AFLP markers linked to parasitism loci have been identified. These genes are being mapped and isolated. They will be engineered into avirulent nematode lines to confirm their function. However, routine transformation of soybean cyst nematodes to express GFP as a marker under the control of a muscle-specific promoter is still unpredictable. A strategy for nematode transformation by microinjection of DNA constructs into the male testis has been devised. Transformants are mated with virgin females, and their progeny is screened under the microscope for expression of GFP (Opperman and Bird, 1998). Theoretically, all nematode parasitism genes are potential targets for manipulation.

Toxic Proteins Expressed in Plant Cells

Transproteins toxic to nematodes, but not to plants, can be expressed into tissues and cells fed upon by nematodes. Toxic proteins such as the barnase mentioned above do not come under this heading since their role is to disable plant cells rather than nematodes. Transgene products with a potential to interfere with nematode physiology, such as digestive enzymes or structural proteins of the intestine, are considered here.

Proteinase inhibitors: All living cells contain a variety of proteinase inhibitors to regulate their endogenous proteolytic activity. However, plant organs that accumulate these inhibitors are often protected from pests and parasites because these inhibitors bind strongly, sometimes irreversibly, to the active site of digestive proteinase enzymes (Ryan, 1990; Vrain, 1999). As with insects, proteinase inhibitors in the diet of plant-parasitic nematodes probably bind to digestive proteinases in the gut to prevent protein hydrolysis and absorption of amino acids. The nematodes would then excrete undigested proteins along with their own digestive proteinases, resulting in a net loss of protein.

Research with proteinase inhibitors goes back to the late 1970s, when a resistant line of cowpea was shown to resist several lepidopteran insect pests because of its elevated content of a serine proteinase inhibitor (CpTi). The inhibitor was engineered in tobacco and successfully controlled lepidopteran insect pests (Hilder et al., 1987). Transgenic potato plants expressing this trypsin inhibitor did not support normal development of *M. incognita* or the reproduction of *Globodera pallida* (Hepher and Atkinson, 1992). Cyst nematodes entered the roots, established themselves, and developed but showed a noticeable shift in sex ratio, with five times more males than females in the transgenic roots. The proteinase inhibitor changed the nutritional value of the plants, and their altered diet influenced the sexual fate of the juvenile cyst nematodes. Root-knot nematodes developed normally, but females produced fewer eggs in the transgenic roots.

Michaud et al. (1996) found major cysteine proteinase activity in three species of root-knot nematodes. A cysteine proteinase inhibitor from rice, oryzacystatin I (OC-I), completely inhibited the proteolytic activity of all stages of *M. hapla*. The tighter the enzyme-inhibitor complex, the more effective the inhibitor; however, OC-I did not bind with high affinity to the proteinases of *M. incognita* and *M. javanica*. Rice also produces OC-II, another cystatin which proved effective against these two nematode species, demonstrating a great specificity between particular plant inhibitors and specific nematode proteinases. Potato cyst and soybean cyst nematodes possess intestinal cysteine proteinases that are, like those of root-knot nematodes, sensitive to OC-I (Koritsas and Atkinson, 1994; Lilley et al., 1996). The proteinase inhibitor gene was modified by site-directed mutagenesis to produce OC-IΔD86. A single amino acid deletion near the binding site improved the conformation of the protein and made it bind with increased avidity to the proteinases of the potato cyst nematode (Urwin et al., 1995). Root-knot and sugarbeet cyst nematodes did not develop normally in transgenic *Arabidopsis* roots expressing the wild rice inhibitor OC-I or the variant protein OC-IΔD86. Fe-

male nematodes in transformed and untransformed roots develop initially at the same rate, but females in roots expressing the mutated protein remained smaller and produced very few eggs (Urwin et al., 1997a).

Two proteinase inhibitors can be fused into a single protein. CpTi, the original serine proteinase inhibitor from cowpea, and OC-IΔD86 were joined by peptide linkers refractory or susceptible to proteolytic cleavage. Cleavage was not essential for the activity of either inhibitors because papain (a cysteine proteinase) and trypsin (a serine proteinase) were inhibited by the fusion proteins. Surprisingly, the inhibitors from the cleavable fusion protein were not ingested by the sugarbeet cyst nematode feeding on transgenic *A. thaliana*. The inhibitors from the other fusion protein, a 23-kDa noncleavable protein, were also not found in the sugarbeet cyst nematode, possibly because the size of the fusion protein was too large to be ingested. However, both fusion proteins still had profound effects on female development and fecundity (Urwin et al., 1998). Transgenic rice expressing low levels of OC-IΔD86 was also resistant to *M. incognita* (Vain et al., 1998).

Bacillus thuringiensis sporulation proteins: B. thuringiensis (Bt) is a common soil bacterium that accumulates large protein crystals when it sporulates. There are more than 100 strains of Bt that crystallize many variant proteins. When these Bt proteins are on plant parts ingested by insects, they dissolve in the insect midgut and are processed by digestive proteases into smaller polypeptides. These polypeptides bind to receptors and disrupt the insect midgut membranes (Vadlamudi et al., 1995). Osmotic balance is lost, the cells of the midgut membrane lose their function, and the insects stop feeding and die. Many strains of Bt are not toxic to insects so the bacteria must rely on other hosts to multiply and disseminate. Several Bt strains have been found to produce polypeptides that kill bacterial feeding nematodes (Borgonie et al., 1996a, 1996b, 1996c; Feitelson et al., 1992; Mena et al., 1996, 1997). Toxicity of Bt strains against nematodes is as specific as with insects, suggesting a similar mode of action. However, the molecular structure and physiology of the intestine of nematodes is too poorly understood to predict that plant-parasitic nematodes will be affected by these bacterial proteins.

Lectins: Lectins are proteins that bind to carbohydrates with high specificity. These proteins accumulate in large quantities in many seeds and in other storage organs of plants. We do not know the role of lectins in the physiology of plants. They may be involved in transporting carbohydrates, cell wall elongation, cell-cell interactions, or growth regulation. Lectins may be the instrument that recognizes receptors in membranes, have enzymatic functions, or may simply be storage proteins. Because most lectins are toxic to animals, including insects and humans, we now think that lectins may also act as defense proteins (Chrispeel and Raikhel, 1991; Peumans and van Damme, 1995). A few lectins are reputed to be nontoxic to mammals but toxic to certain insects (Gatehouse et al., 1995). A mannose-binding lectin engineered in various crops is toxic to aphids, plant hoppers, and several nematodes including root-knot, root lesion, and potato cyst nematodes (Anwar and McKenry, 1998; Boulter et al., 1990; Burrows et al., 1998).

Cholesterol oxidase: The enzyme cholesterol oxidase represents a new class of insect control proteins. A screening program at Monsanto to find proteins toxic to insect pests, nematodes, and other parasites and pathogens has yielded many useful proteins, including a cholesterol oxidase from *Streptomyces* culture medium that is extremely effective against cotton boll weevil and other insects (Greenplate et al., 1995; Purcell et al., 1993). One ppm of cholesterol oxidase in their diet kills 50% of weevil larvae and severely retards the development of the other 50%. This toxicity is roughly equivalent to that of Bt toxins. Cholesterol oxidase disrupts the insect gut by enzymatic oxidation of cholesterol in the cellular membranes. Effects against nematodes have not been published.

Outlook

This review of current genetic engineering studies in nematology suggests that numerous natural and synthetic resistance genes soon may be available against nematodes. Tomorrow, genomic and proteomic sciences will identify many novel nematode and plant genes and their encoded protein products, thus creating opportunities to interfere with the establishment and maintenance of host-parasite relationships. With the accelerating pace of breeding natural resistance genes, especially against cyst and root-knot nematodes, it becomes obvious that both natural and synthetic resistance against nematodes will have a prominent place in agriculture.

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