Pathogenicity factors of *Meloidogyne* and host plant response

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Sedentary endoparasitic root-knot nematodes, genus *Meloidogyne*, are among nature’s most successful parasites infecting more than 2000 plant species. They are a tremendous threat to crop production world-wide (Sasser, 1980; Sasser & Freckman, 1987). Understanding the plant-nematode interactions is of major importance in order to elaborate new control strategies, since treatment of infected fields with nematicides is expensive and their use is restricted because of their toxicity to humans and the environment.

These sedentary endoparasitic nematodes interact with their hosts in a most fascinating way. They are able to induce the redifferentiation of root cells into nematode feeding sites (NFS) (Gheysen *et al.*, 1996). Plant invasion by root-knot nematodes begins with the infective second stage juveniles (J2), which penetrate the root tip just behind the root cap, then migrate intercellularly along the vascular cylinder searching for the procambial cells that will be changed into giant feeding cells. The J2 then undergo three moults to develop into adults. Females remain sedentary, producing large egg masses and galls. Males migrate out of the plant. The mode of parasitism of root-knot nematodes is very similar to what is observed in the case of the phylogenetically closely-related cyst nematodes, which are also sedentary endoparasites infecting the root tissues. The main difference in parasitism between cyst and root-knot nematodes is that cyst nematodes migrate intracellularly through the root tissue. For both groups of nematodes, the success of parasitism thus largely depends on the efficiency with which infective juveniles penetrate and invade the root tissues. Therefore, these nematodes need some means to break down cellulose to help them migrate through root tissues.

Nematode growth and reproduction depend on the establishment of the NFS. Cytological observations indicated that these NFS are multinucleate with enlarged nuclei and nucleoli. Compared to normal cells, NFS also show an increase in cytoplasmic density, a loss of normal vacuolation and a proliferation of cell organelles. Another characteristic feature of these structures is development of cell wall ingrowths, typical of transfer cells (Jones, 1981). These cell wall ingrowths increase the surface area of the associated membrane and thus facilitate the import of elaborated photosynthates, minerals and other metabolites.

Depending on the nematode species, the initial feeding cell develops into either a syncytium (for cyst nematodes, such as *Heterodera* and *Globodera* spp.) or a system of giant cells (for the root-knot nematodes, *Meloidogyne* spp.) (Jones, 1981). Syncytia result from cell fusions after cell wall dissolutions between the initial cell on which the nematode starts feeding and an increasing number of neighbouring cells. Up to 200 cells can be incorporated in a large syncytium. Conversely, giant cell formation is the result of repeated nuclear divisions of the initial feeding cell without cytokinesis. Each root-knot nematode triggers the development of five to seven giant cells, each containing as many as 100 nuclei, which have undergone vast endoreduplication (Wiggers *et al.*, 1990). Because *Meloidogyne* species can induce similar giant cells in several thousand host species, they probably interact with some fundamental key steps of the plant cell cycle (Niebel *et al.*, 1996). In addition, root-knot nematode development is accompanied by divisions of cortical cells around the NFS, giving rise to a typical root-knot gall.

Nematodes withdraw food from NFS until the completion of their life cycle. However, it is not yet understood how these nematodes cause such alterations, but it is suspected that glandular secretions injected into plant cells interact directly or indirectly with the plant nuclear genome (Hussey, 1989).

In our laboratory, we focused on two aspects of the plant-nematode interactions, the nature of the salivary
secretions that the nematodes inject during parasitism and the host response to the initiation of the giant cells.

**Molecular responses of the plant**

To identify new genes and obtain a more comprehensive view of the molecular mechanisms underlying the induction and maintenance of NFS, a promoter trapping strategy was developed with a promoterless β-glucuronidase (GUS) construct introduced randomly into the *Arabidopsis* genome via Agrobacterium T-DNA transformation (Bechtold *et al.*, 1993; Bouchez *et al.*, 1993). Using the T-DNA-tagged *Arabidopsis* lines obtained by INRA Versailles, we have screened 20 000 lines and isolated 200 lines showing GUS expression in galls. The first results we obtained with this strategy are encouraging since we have isolated 23 genes homologous to genes involved in metabolism, signal transduction, biotic or abiotic stress responses and cytoskeleton organisation. Moreover, we have recently identified, for the first time, an upregulated nematode-responsive gene that is essential for the early steps of giant cell formation (Favery *et al.*, 1998). Its expression pattern is similar to that of key regulators of the cell cycle, but it is not observed with cyst nematode infections. Later in NFS development, this gene is induced by both root-knot and cyst nematodes. This gene encodes a protein similar to the D-ribulose-5-phosphate 3-epimerase (RPE) (EC 5.1.3.1), a key enzyme in the reductive Calvin cycle and the oxidative pentose phosphate pathway (OPPP). Quantitative RT-PCR showed the accumulation of *RPE* transcripts in potato as in *Arabidopsis* NFS. Homozygous *rpe* plants have a germination mutant phenotype that can be rescued in dwarf plants on sucrose-supplemented medium. OPPP may provide NADPH and sugar intermediates necessary for NFS induction and/or maintenance. During root development, this gene is also expressed in meristems and initiation sites of lateral roots. These results suggest that a common key regulator is involved in the formation of giant cells and these organs and confirm the previous cytological observations indicating that a complex redifferentiation process occurs in NFS.

Since 100% NFS promoter specificity is predicted not to exist, promoters will be deeply analysed to develop nematode resistant transgenic plants. Promoter sequences that bind nuclear proteins obtained from NFS will be identified by DNA-protein interaction analysis (gel retardation assays, footprinting, etc.). Artificial chimeric promoters (containing NRE) will be produced, fused to GUS and/or green fluorescent protein (GFP) and transformed into *Arabidopsis thaliana*. It will be particularly important not only to check nematode feeding site expression but also to analyse in detail expression outside the NFS under as many environmental conditions as possible. The most specific promoter(s) will be taken to drive expression of selected genes specifically in NFS. Finally, the functionality of these promoters will be tested in other plants, including tomato and rapeseed.

**Nematode stylet secretions**

On the nematode side, we focused our efforts on the analysis of the protein content of purified stylet secretions. In order to analyse the content of stylet secretions by 2D-gel electrophoresis, a procedure has been set up for large scale production of *Meloidogyne* infective juveniles and purification of stylet secretions. In this procedure, stylet secretions from the juveniles were induced by incubation in resorcinol. Analysis of these samples on 2D-gels showed the presence in the secretions of about 40 proteins of between pI 5 and 7.5. Staining the 2D-gels with Coomassie blue or silver nitrate both showed that no contaminant protein is extracted with this procedure. To our knowledge, this is the first time stylet secretions have been efficiently purified from *Meloidogyne* juveniles. The amount of proteins we obtained allowed direct analysis of the secretions by internal sequencing of the major proteic spots. Protein internal sequences were compared with identified sequences from databanks.

Three groups of proteins were identified. The first group is composed of proteins involved in the cytoskeleton, such as tropomysin or troponin. The second group is composed of proteins known to have functions in metabolism regulation, such as ATP synthase β chain or myosin regulatory light chain (MRLC). Finally, some proteins are known to have multiple functions, including cell cycle regulation, such as calreticulin and 14-3-3 protein.

From the protein sequences, degenerated oligonucleotides were designed, that allowed the cloning of the MRLC, calreticulin, 14-3-3, troponin and tropomyosin cDNAs. Studies are now underway in order to check the expression of these proteins in the pharyngeal glands of the nematode. Promising results have been obtained with calreticulin, whose expression in the subventral pharyngeal glands of second stage juveniles has been demonstrated by *in situ* hybridisation and immunolocalisation.

The isolation of cellulase genes in root-knot nematode represents a good example of the candidate gene strategy. By using conserved regions of nematode, fun-