# Investigating gene expression in nematode-infected roots:

V.C. Blok, M.R. Armstrong, B. Banks, P.R.J. Birch, G. Bryan, L. Castelli, J.T. Jones, A. Kumar, M.S. Phillips, A. Purvis, G. Ramsey, R. Waugh & J. Wishart

Potato cyst nematodes (PCN) cause significant economic losses in potato production in the UK (>£50million/annum, Parker, 1999). A recent survey has shown that the distribution of the two species of PCN (*Globodera rostochiensis* and *G. pallida*) found in the UK is increasing with the latter becoming more prevalent (Minnis *et al.* 2000). To control PCN, crop rotations, resistant cultivars and nematicides are used. The *H1* resistance gene has been incorporated into various commercial varieties and is used effectively to control *G. rostochiensis.* However, as a consequence *G. pallida*, for which this resistance gene is not effective, has become more prevalent. No single major resistance gene against *G. pallida* has been incorporated into UK varieties. Several varieties with polygenic resistance to *G. pallida* are available but these are not widely grown in the UK. Finding new strategies to control *G. pallida* is of increasing importance. Research at SCRI, which is funded through SEERAD and EU collaborative projects, is addressing this using different genomic approaches.

Incorporating novel resistance into UK varieties, including the recently isolated *Hero* gene, which gives resistance to *G. rostochiensis* and *G. pallida* in tomato, is underway at SCRI. In addition, understanding the molecular events underpinning resistant responses to pathogens is expected to offer opportunities for enhancing the protection of crops and provide alternative resistance to economically important pests and diseases.

Pathogens vary in their capacity to induce disease depending on the genetic constitution of the host. When the pathogen expresses a specific avirulence (*avr*) gene and the plant contains the cognate resistance (*R*) gene, a form of localised programmed cell death called the hypersensitive response (HR) can occur at the site of infection, inhibiting the pathogen. Typical responses associated with the HR include protein phosphorylation, production of reactive oxygen species, ion fluxes and G-protein signalling, all of which contribute to the activation of defence-related genes. Defence-related genes have been shown to encode low molecular mass antimicrobial compounds called phytoalexins, hydrolytic enzymes such as chitinases and endoglucanases, new cell wall compounds, systemically accumulated proteinase inhibitors and other defence proteins of known or unknown function. In nematode-plant interactions, the classic *R*/*avr* rapid HR is seen with avirulent root knot nematode juveniles (*Meloidogyne)* within 6 hours of invasion of tomato roots of plants with the single dominant *Mi* resistance gene. Strong induction of gene expression is observed using the cDNA-AFLP technique ( Fig. 1).

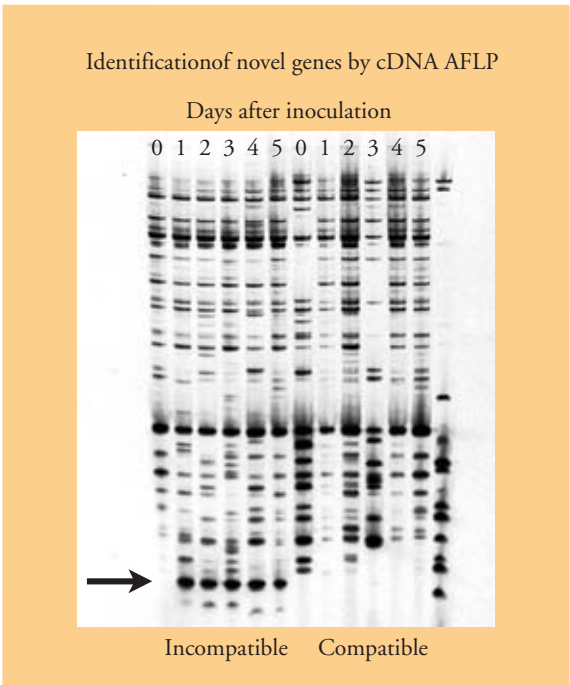


**Figure 1** Electrophoresis pattern from cDNA AFLPs showing induction of gene expression in incompatible interaction with Meloidogyne javanica infected tomato with Mi resistance gene but not in uninfected or infected compatible interaction

However, for plant parasitic cyst nematodes the resistant reaction is more typically a delayed necrotic response. Invasion and intracellular movement through the root cells causes wounding and damage; the nematodes eventually settle and begin to modify the host root cells to produce enlarged and metabolically highly active feeding sites. Histological examina-

tion of roots shows that, initially, the incompatible/resistant interaction appears like a compatible interaction. However, after several days the feeding site development is restricted and necrosis surrounding the developing feeding site is often observed. Development of invasive stages of the nematodes is delayed and, typically, development of females is poor with males predominating in the adult stage thus limiting the reproductive potential of the pest (Fig 2). The histological responses of the host to cyst nematodes with a single major resistant gene or polygenic resistance have thus far been found to be similar. Currently, additional sources of resistance to *G. pallida* in the Commonwealth Potato Collection are being examined histologically to determine if different mechanisms of resistance can be found.

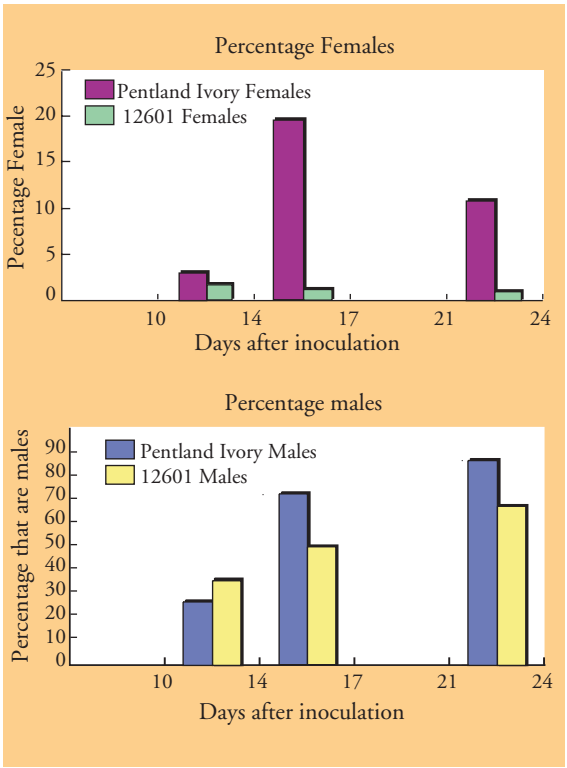


**Figure 2** Percentage of females A) of males B) at different time points in a susceptible (Pentland Ivory) or a resistant (12601) host.

We are examining changes in gene expression in infected potato roots resistant to PCN with the aim of identifying components of the plant response pathways. This is being done in parallel with similar studies comparing responses in potato to other nematodes (i.e. *Meloidogyne chitwoodi*) and to the foliar pathogens *Phytophthora infestans* and *Erwinia carotovora*. By contrasting these different pathosytems of potato during incompatible and compatible interactions, candidate genes for potential exploitation in developing broad-spectrum resistance are being identified. Host responses specific to incompatible interactions have not been well characterised at the molecular level. Induction of genes typically associated with plant defence responses, such as peroxidases, chitinases, lipoxygenases and proteinase inhibitors have been observed in incompatible interactions but these have also been observed to accumulate in compatible responses. We are attempting to identify specific responses in resistant interactions.

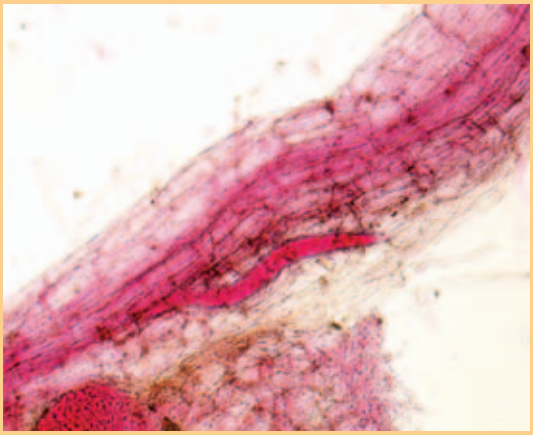

**Figure 3** Feeding PCN juvenile nematode in potato root tissue stained with acid fuchsin.

One approach we have used is a PCR-based method, suppressive subtractive hybridisation (SSH), which enriches for rare, differentially expressed transcripts. The enriched libraries were sequenced and database searches were conducted using FASTA, BLASTN and BLASTX on *c.* 2000 sequences. Many of the sequences obtained, when compared to those in databases, were similar to either plant or nematode expressed sequence tags (ESTs). However, many showed no reliable sequence similarities. In some experiments, a significant number of sequences (25-72%) were similar to potato cyst nematode ESTs, indicating that considerable enrichment for rare sequences had occurred, as nematodes comprise a tiny proportion of the root biomass (Fig 3). These include genes coding for proteins expressed in the oesophageal and dorsal glands (Fig 4), such as β-1,4-endoglucanase, which is thought to play a role in root invasion and feeding site development and is believed to have been acquired by horizontal gene transfer from bacteria. Other genes code for structural proteins such as 40S and 60S ribosomal proteins, calponin, and other functionally interesting genes such as surface-expressed fatty acid binding proteins.

**Figure 4** In situ hybridisation of preinvasive PCN juvenile nematode with cellulase gene.

During the development of the feeding site of PCN, cell walls degrade giving rise to a multinucleate, highly metabolically active syncytium. Consistent with these changes in cell wall structure, xyloglucan endo-transglycosylase, an important component in cell wall metabolism has been identified in SSH libraries from incompatible interactions between different potato genotypes and PCN and RKN. Interestingly, from the compatible interactions, plant-derived β-1,4-endoglucanases, similar to those also produced by nematodes, were identified. From an incompatible interaction, germin-like proteins were identified. These proteins are believed to be involved in defence responses to fungal pathogens (Lane 2002).

In an alternative approach to identify gene expression differences in resistant and susceptible roots, the 10 most susceptible and resistant clones from a F1 cross of PCN resistant and susceptible parents were bulked. AFLPs performed on these bulks have revealed numerous AFLP bands that segregate with PCN resistance. Expressed sequences that also segregate with PCN resistance may be involved in a resistance pathway. Using the same bulks, cDNA-AFLPs were performed and bands present in the resistant and absent in the sensitive bulks were excised, cloned and sequenced. PCR primers were developed to each of these sequences and used to find single nucleotide polymorphisms (SNPs) in the parents of mapping populations. Having verified that the SNP markers segregate with the resistance phenotype, the map locations of the SNPs are being determined.

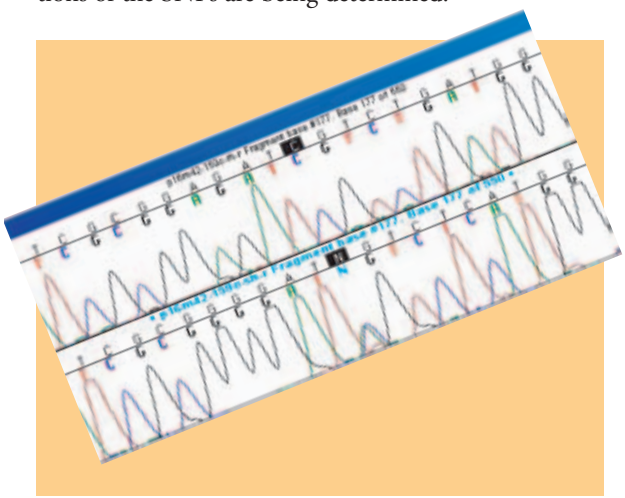

**Figure 5** Sequence of a cDNA from a susceptible and resistant host showing a SNP.

Through these approaches candidate genes that may have utility both as markers to assist selection in breeding programs, and which can be manipulated through genetic engineering to provide novel sources of resistance, are being found. In the future providing novel resistance to important crop pathogens which leads to reduced reliance on pesticides is in line with environmentally sound and sustainable agriculture objectives.

References

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Parker, B. (1999). "Living with the enemy." Eyewitness 7:12-13.