2015 RESEARCH FINDINGS
in the School of
VETERINARY & LIFE SCIENCES

Green worm: Root lesion nematode Pratylenchus thornei *isolated from wheat, treated with fluorescein isothiocyanate (FITC) to check for uptake of external solution containing dsRNA.*

BULLETIN
3.01
Crop Production & Biosecurity

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The (nematode) worm has turned for better crop plants

Nematodes that attack plant roots are the hidden enemies of many different crop plants, including wheat, barley, sugarcane, legumes, potatoes, tomatoes, brassicas and almost all horticultural plants.

Nematodes cause an estimated US$120 billion in crop losses worldwide, which translates into lost food production that would feed 350 million people. Most plant nematodes are soil pests: they reduce plant water and nutrient use, and enable entry of other root pathogens. This translates to stressed plants and reduced yields and quality.

Despite their economic importance, study of plant parasitic nematodes has been largely neglected, until now. The free-living nematode *Caenorhabditis elegans* was chosen as a model animal to understand the molecular basis of many animal properties, and its genome is now the best understood of any multicellular organism. This resource has enabled us to compare the genes of *C. elegans* with those of plant parasitic nematodes and so identify vital genes needed for metabolism or plant parasitism, providing new target genes for nematode control.

Methods and results

Using Next Generation Sequencing, we have sequenced the expressed genes (transcriptomes) of root lesion nematodes (*Pratylenchus* spp) that attack wheat, barley, and sugarcane, and beet cyst nematode (*Heterodera schachtii*) that attack brassicas and beets (Figures 1 and 2). We have also used other data available for economically important cyst and root-knot (*Meloidogyne* spp) nematodes to identify targets for their control.

We fed juvenile plant parasitic nematodes double stranded RNA (dsRNA) of target genes, using a process called gene silencing or RNA *interference* (RNAi).

FIGURE 2 Cysts of beet cyst nematode (*Heterodera schachtii*) isolated from brassica crop: each contain hundreds of eggs

FIGURE 1 Collecting beet cyst nematode cysts in a farmer's brassica crop north of Perth

This method makes use of a natural process, in which nematodes will destroy their own mRNA if it has the same sequence as the introduced dsRNA. If the targeted gene is vital for nematode survival or parasitism, then the nematode will die.

We compared promising candidate target genes by screening the effects of feeding nematodes with these target gene sequences *in vitro*. The dsRNA of the most effective candidate genes for nematode control was then expressed in transgenic plants (*in vivo*) (Figure 3). When nematodes ingest cell contents from these plants, if a vital nematode gene is silenced, the plant effectively becomes resistant to nematode attack (Figure 4). Using this method, a reduction of nematode infection of up to 90% or more can be achieved in different transgenic plant events (Figure 5). Similar results have been obtained for silencing target genes in other important nematode pests (root knot and root lesion nematodes), showing that this process of 'Host Induced Gene Silencing' can provide a new genetic form of resistance to plant parasitic nematodes.

Conclusions and recommendations

We have shown that Host Induced Gene Silencing is widely applicable and can be used to control the three major groups of nematode pests (root knot, cyst and root lesion) of crop plants. We are also investigating how we can translate this technology into commercial reality, either via transgenic plants, or via alternative mechanisms. ■

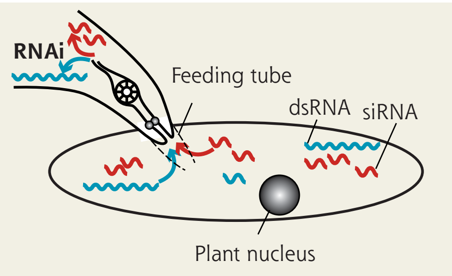

FIGURE 3 Nematode ingestion of dsRNA from a host cell (dsRNA — double stranded RNA, siRNA — small interfering RNA)

FIGURE 4 Challenging transgenic plants in the glasshouse with beet cyst nematodes, and counting cysts about 5 weeks after inoculation with J2 juveniles

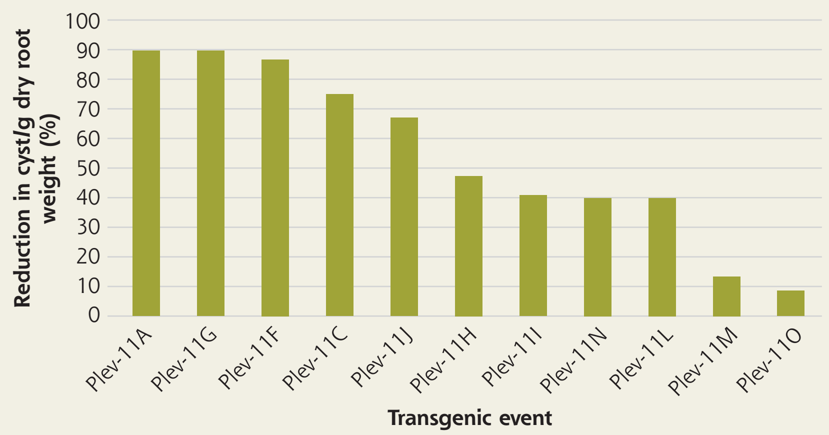

FIGURE 5 Results of screening for plant resistance to the beet cyst nematode. Up to a 90% reduction in nematode reproduction has been obtained

More information

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5 Figure 3 redrawn from: Bakhetia, M. *et al*., (2005). RNA interference and plant parasitic nematodes. *Trends in Plant Science* **10**, 362–367.

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