Characterisation of effectors from the potato cyst nematode *Globodera pallida*

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AIMS

Identify effectors from *G. pallida*

Identify effectors that suppress host defence responses

Figure 1 (above): invasive stage J2 of Globodera pallida.

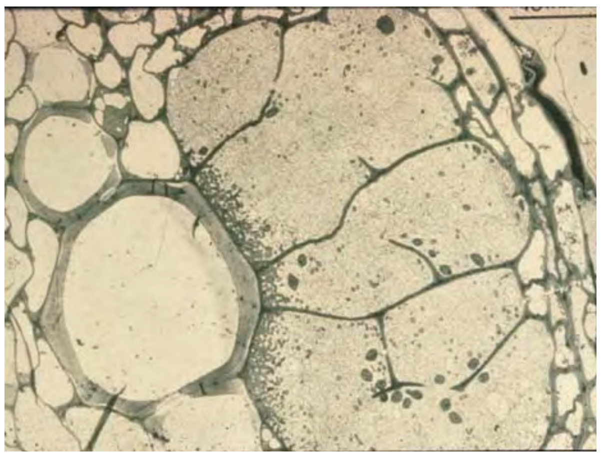


Figure 2 (right): cross section of a syncytium induced by G.pallida *in potato roots.*

Plant parasitic nematodes are damaging pathogens

The most economically important nematodes are sedentary biotrophic pathogens, including root knot and cyst nematodes

Potato cyst nematode, *Globodera pallida* (Figure 1), is a particular problem within the EU

Cyst nematodes induce the formation of a syncytium (Figure 2) in their hosts

Host defences must be suppressed in order to protect the feeding site and developing nematode.

This is thought to be achieved using effector proteins secreted from the oesophageal gland cells.

Effectors have a range of other roles, including facilitating migration and induction of the feeding site

Over expression of effectors in plants

Pioneer effectors have been identified from the *G. pallida* genome and transcriptome sequences using a bioinformatic approach. Some exist as large gene families

Potato and *Arabidopsis* have been transformed with a total of 21 effectors and with control constructs

A wide range of potato transformation efficiencies has been observed (Figure 3). This may be due to an effect of the nematode gene on the plant

Phenotypic effects have been observed in *Arabidopsis* including effects on growth rates (Figures 4 and 5)

Plants expressing effectors that suppress host defences may be compromised in their ability to respond to pathogens

High throughput assays are therefore being developed that will allow identification of transgenic plants that are more susceptible to pathogens

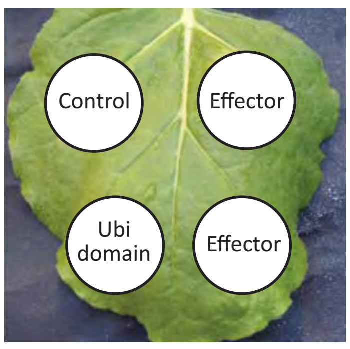
Figure 3 (above left): variation in potato transformation efficiency due to effector expression
Figure 4 (above right): severely stunted growth in Arabidopsis expressing a nematode effector
Figure 5 (below): Arabidopsis transformed with different effectors show a range of different growth rates

A *G. pallida* ubiquitin extension protein suppresses host defence responses

Figure 6 (above left): The ubiquitin extension protein suppresses PTI (PAMP triggered immunity) induced by INF1. PTI is not suppressed by the UBI domain alone

Figure 7 (above centre): The ubiquitin extension protein and the ubiquitin domain alone suppress ETI (effector triggered immunity) induced by R3a and Avr3aKI

Figure 8 (above right): ETI induced by Gpa2 and AvrGpa2 is not suppressed by any of the ubiquitin extension protein or ubiquitin domain constructs



Schematic diagram showing position of constructs in images above. Additional spot in Figure 8 is an additional (GFP) control

G. pallida secretes a ubiquitin extension protein into hosts from the dorsal gland cell. This protein consists of a ubiquitin domain and a short C-terminal extension

The full length effector suppresses INF1 induced PTI in *Nicotiana benthamiana* whereas the ubiquitin domain alone does not (Figure 6)

The full length effector, and the ubiquitin domain alone, suppress ETI induced by a combination of R3a and Avr3aKI (Figure 7). However, ETI induced by Gpa2 and RBP1 (AvrGpa2) is not suppressed by either protein (Figure 8)

In current work we are investigating the host signalling pathways targeted by the ubiquitin extension protein

SUMMARY

A panel of transgenic plants expressing *G. pallida* effectors have been produced and will be phenotyped in detail

A *G. pallida* ubiquitin extension protein that suppresses host defences has been characterised