

# Molecular Characterization of the Expansin gene in *Globodera pallida* and *Globodera ellingtonae*



Potato cyst nematodes (PCN) are serious pests of potato worldwide. *Globodera pallida* is a quarantine pest in the United States and found only in Idaho. *Globodera ellingtonae*, a new species of cyst nematode has a biotrophic relationship with the host plant and can reproduce on potato. Expansin genes present in nematodes and in potato are associated with several plant metabolic and parasitic functions. For nematodes, expansin has been shown to suppress defense responses, and may be important in syncytium formation. In plants, expansin is crucial to cell growth, emergence of root hairs, and other developmental processes where cell wall loosening occurs. Therefore, the identification and experimental validation of this target gene could help to develop novel management practices against PCN.

## Objectives:

- To locate the putative effector expansin gene (*Gp-exp* and *Ge-exp*) in both nematode species by *in situ* hybridization
- To verify the expression of the expansin gene (*Stexpa1*) in susceptible and resistant potato after nematode infection
- To investigate whether nematode expansin genes are regulatory mimics which can modify host plant cell wall structure

## Methods:

### Identification of *Gp-exp*, *Ge-exp* and *Stexpa1* genes

Database searching – Design specific primers  
RNA extraction from J2 (Plus Micro Kit Rnase-Free Dnase set)  
Reverse transcription (Invitrogen Superscript II) – Cloning (Topo TA cloning Kit)



### Locate the transcript from *G. pallida* and *G. ellingtonae* J2

*In situ* Hybridization



### Fluorescent staining

J2 stained with PKH26 red Fluorescent Cell Linker



### Inoculate potato varieties

Desiree and Innovator potatoes were infected with 2000 *G. pallida* and *G. ellingtonae* J2 at different time points: 24 h, 2 and 7 days

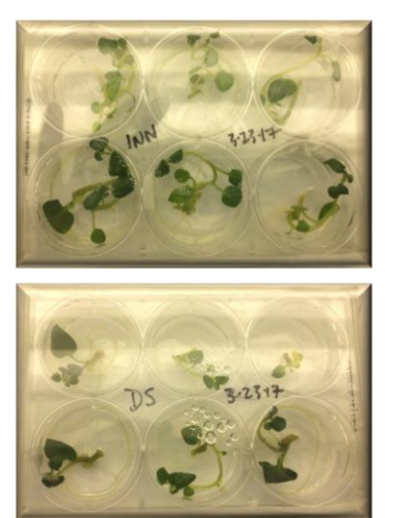
### Identification of *expa1* gene from two potato varieties

Database searching – Design specific primers



### RNA extraction from *in vitro* potato roots

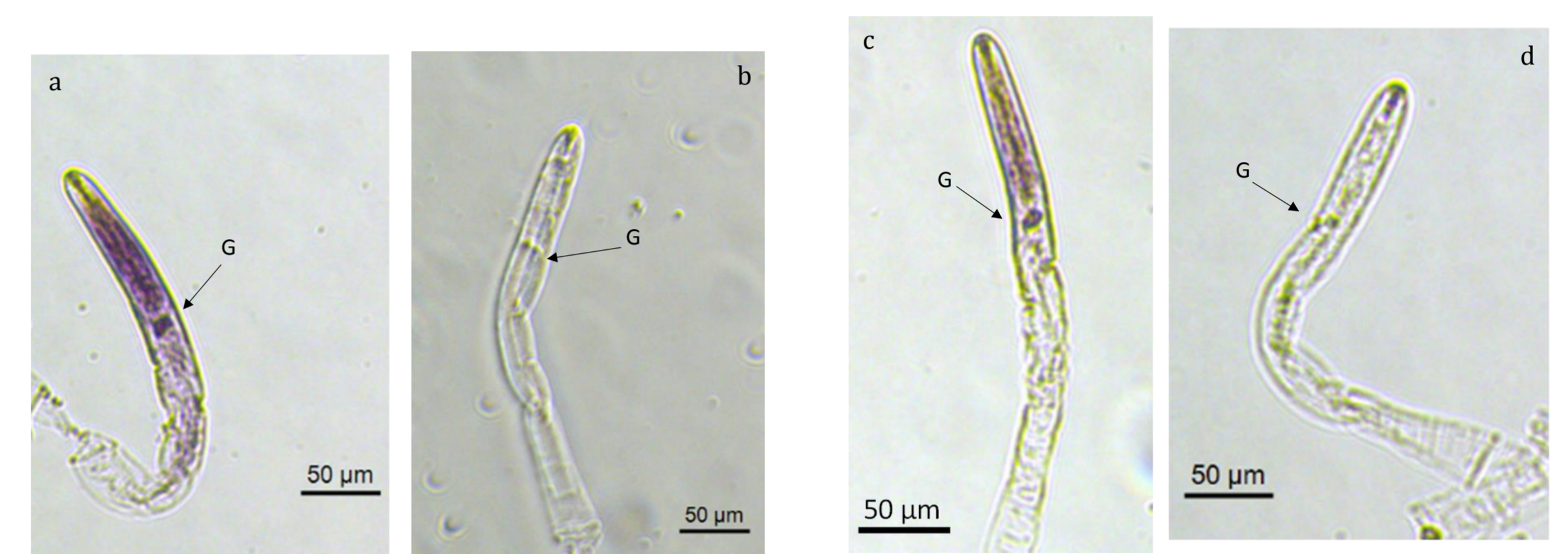
RNeasy Mini Kit



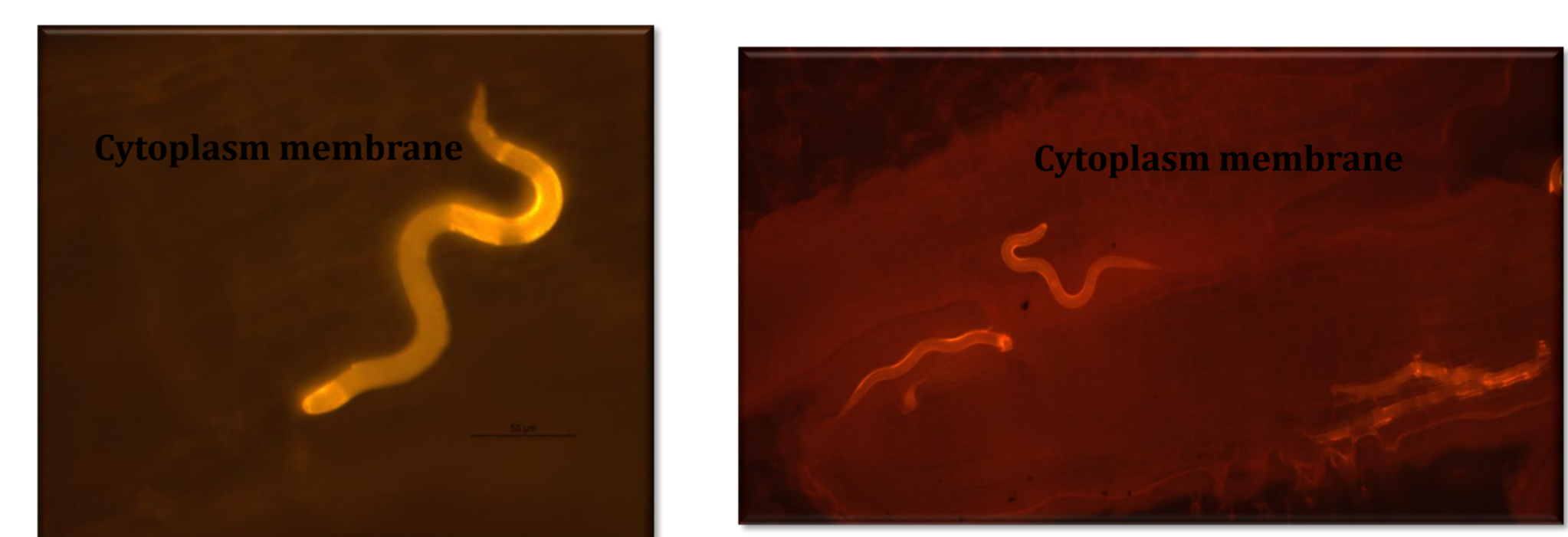
### Study expression of target genes

Syber Green PCR master mix comparative CT method  
Applied Biosystems 7500 fast real time

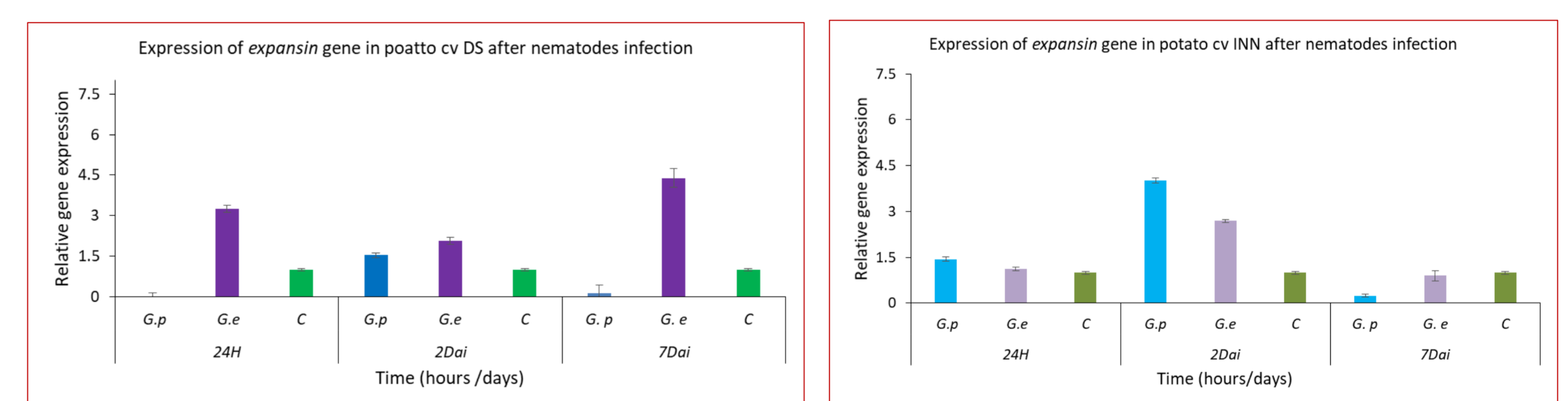
## Results:



**Fig. 1** *Globodera pallida* and *G. ellingtonae* second-stage juveniles (J2) sections hybridized with digoxigenin-labeled sense and antisense cDNA probes derived from the *Gp-exp* (a and b) and *Ge-exp* (c and d) genes.



**Fig. 2** *Globodera pallida* J2 and *Globodera ellingtonae* inside the potato roots stained with PKH26



**Fig. 3** Evaluation of relative expansin gene from susceptible (Desiree) and resistant potato cultivars (Innovator) after *Globodera pallida* and *Globodera ellingtonae* J2 infection.

## Conclusions:

- Highly active transcription of the *Gp-exp* and *Ge-exp* in the subventral oesophageal glands from the J2 was detected in both *G. pallida* and in *G. ellingtonae*.
- Expansin gene is a putative effector gene in *G. pallida* and *G. ellingtonae*
- Differential expression of *Stexpa1* gene was detected in Desiree and Innovator potato varieties after the infection of J2 *G. pallida* and *G. ellingtonae*
- The putative mimicry effect of the expansin gene to modify host plant cell wall structure can lead to the development of novel approaches for the management of *G. pallida* and *G. ellingtonae*