VIGS for functionally analysing novel defence genes in potato

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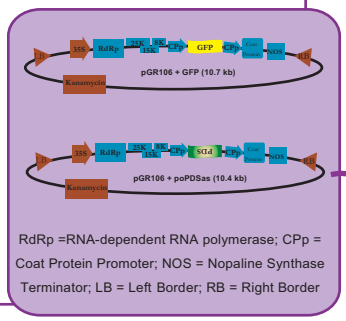
Introduction

The hypersensitive response (HR) is an inducible form of programmed cell death that is one of the first and most effective plant responses that restricts the growth of avirulent pathogens. Evidence suggests that HR is fundamental to resistance against the oomycete *Phytophthora infestans* which causes severe damage to potato crops. Novel and established defence genes, from both potato and *Arabidopsis*, need their putative role in defence responses against *P. infestans* in potato examined. Many viral vectors induce a dsRNA-mediated, sequence specific defence mechanism that targets the viral genome and any homologous RNA, known as virus-induced gene silencing (VIGS). Viral vectors are successfully used to study gene function in other solanaceae family members. The VIGS-inducing potential of a recombinant potato virus X (PVX) expression vector is examined in potato, aiming to develop a VIGS-based functional bioassay for defence genes in potato (Thomas *et al.*, 2001)

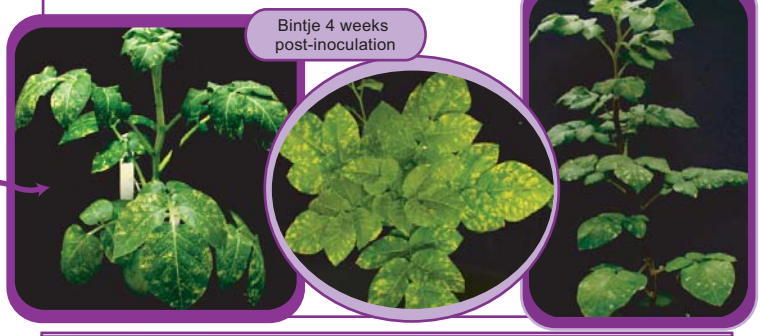
Potato Virus X Vector

- Replicates sufficiently in potato for PVX-based vectors to be successfully utilised for expression of inserted host sequences of interest.
- Hypothesised to replicate its RNA genome sufficiently to trigger post-transcriptional gene silencing of inserted plant sequences of interest.
- Initially tested vector with *GFP*, as a control and a 400bp fragment of the reporter Phytoene desaturase (*PDS*) gene from potato, cloned in anti-sense.



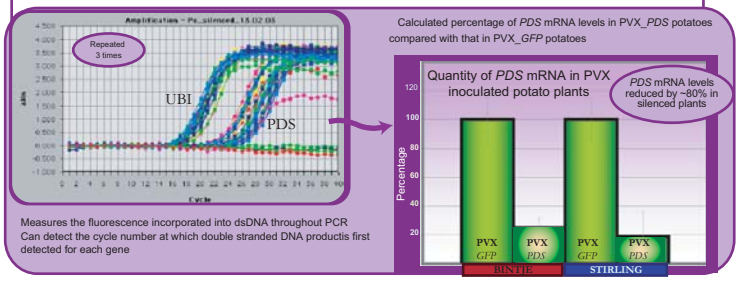
PDS Silencing in Tetraploid Potatoes

- Modified PVX-binary vectors transformed into *Agrobacterium*.
- Pressure infiltrated into 3 week old *P. infestans* resistant potato cultivar Stirling and susceptible cultivar Bintje.



Quantifying PDS mRNA in Potatoes

- Quantified *PDS* mRNA levels in pGR106 inoculated plants with respect to the constitutively expressed control, ubiquitin (*UBI*).
- cDNA synthesised from RNA extracts of whole Stirling and Bintje leaves at 1 month and 2 months post-inoculation
- Used quantitative real time PCR with primers that amplified short region of *UBI* or region of *PDS* excluded from PVX vector



Candidate Defence Genes for VIGS

Now silencing has been demonstrated in *P. infestans* resistant and susceptible potatoes, next aim is silencing defence genes of interest.

CathB Discovered by suppression subtractive hybridisation (SSH) of cDNA from *P. infestans* challenged resistant and susceptible potatoes at the SCRI. Cathepsin B reported as essential mediator of death receptor-triggered and caspase-initiated tumour cell apoptosis.

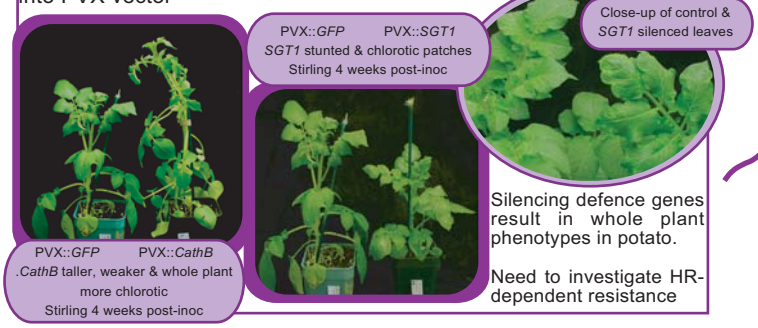
CathK Also discovered by SSH at the SCRI. Encodes a conserved cysteine protease domain and an uncharacteristic C-terminal granulin domain. Granulins bind specific receptors to regulate cell growth and division.

ADR1 Identified from activation-tagged *Arabidopsis* mutant at University of Edinburgh. Constitutive expression results in a broad spectrum resistance phenotype. Protein contains classical *R* gene- like domains however R proteins rarely activate defence pathways before incompatible interaction. Serveral R proteins show similarities with APAF-1 (involved in regulation of mammalian programmed cell death).

SGT1 Originally identified as component of the yeast ubiquitination and kinetochore assembly pathways, however silencing of *Sgt1* in barley revealed its role in resistance against powdery mildew. Positive control for silencing in potato due to its well characterised silenced phenotype in other plant species (Peart et al., 2002).

Phenotype of Defence Gene Silencing

- Thus far, 400 bp fragments of *CathB* and *SGT1*, conserved between potato and *Nicotiana benthamiana*, cloned in anti-sense into PVX vector



Silencing defence genes result in whole plant phenotypes in potato.

Need to investigate HR-dependent resistance

Gene Discovery in *Arabidopsis*

- 10,000 activation-tagged mutants have been screened for altered disease resistance responses to pathogens and activation of a luciferase reporter fused to *PR-1* promoter .
- Genes responsible for phenotype can be isolated by chromosome walking from the inserted activation tag.
- Homologs of these novel genes will also be studied in potato using VIGS e.g. *Adr1*,

T1 generation of a candidate mutant that has lost apical dominance and lost resistance to *Pseudomonas syringae*.

Conclusion and Future Work

- PVX-based vector replicates sufficiently in potato to trigger post-transcriptional gene silencing of host sequences as demonstrated by quantifying the silencing of phytone desaturase (*PDS*) mRNA.
- Use PVX-based vector to silence candidate defence genes, from potato and *Arabidopsis*, in potato
- Investigate whether HR and subsequently resistance have been compromised following challenge with a variety of pathogens

References

Peart JR, Lu R, Sadanandom A, Malcuit I, Moffett P, Brice DC, Schauser L, Jaggard DA, Xiao S, Coleman MJ, Dow M, Jones JD, Shirasu K and Baulcombe DC (2002) Ubiquitin ligase-associated protein SGT1 is required for host and non-host disease resistance in plants. *Proc Natl Acad Sci U S A* **99**:10865-9

Thomas CL., Jones L., Baulcombe DC and Maule AL. (2001) Size constraints for targeting post-transcriptional gene silencing and for RNA-directed methylation in *Nicotiana benthamiana* using potato virus X vector. *Plant J* **25**:417-425