Targeting late blight with gene discovery

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Introduction Potato is the fourth most valuable crop and the highest ranked non-cereal crop. The global area sown to potatoes is increasing at a rate of 4.5 % p.a. and the geographical range in which it is grown is expanding. However, potato has a major disease problem in *Phytophthora infestans*, the cause of late blight. Late blight occurs almost everywhere that potato is grown. *P. infestans* caused the nineteenth century Irish potato famine when Ireland lost over one million people to either starvation or emigration. These impacts can still occur where potato is cultivated as a staple food crop by smallholder or subsistence farmers. On a larger production scale, direct disease losses and costs of disease control are estimated to cost over £50 million p.a. in Britain alone (estimated at £3 bn p.a. globally). Control and management of late blight relies heavily on the regular application of costly control chemicals, but *P. infestans* insensitivity to some chemicals has been recorded. Major genes (*R* genes) for late blight resistance in potato have been used in the past, with limited success, as *P. infestans* has rapidly overcome most major *R* genes.

P. infestans is frequently considered to be a 'fungus' due to its mycelial growth habit, but belongs to a class of organisms known as the oomycetes, more closely related to the wider grouping of the Stramenopiles. This taxonomic affinity gives it many unusual characteristics, compared with the true fungi. The greater significance of this is that many genetic, molecular biological, and plant pathogenesis characteristics that have been determined for the true fungi do not apply, or need to be determined for *P. infestans*.

Recent years have witnessed a burst of activity within the oomycete research community. For example, resources such as expressed sequence tag (EST) databases, limited genome sequencing, large insert DNA libraries, genetic maps, and evolving techniques for functional analysis of genes are now available for *P. infestans*. The high-throughput strategy that characterizes genomic research now allows us to target the genes involved in the molecular interaction of *P. infestans* with potato.

Gene discovery Key targets of SCRI *P. infestans* gene discovery are genes required for the establishment of a successful infection of potato; these genes are likely to be expressed very early in the interaction. The infection strategy of *P. infestans* involves differentiation into as many as five different cell types in the following order (Figure 1): sporangium, zoospore, germinating cyst, appressorium, infection vesicle (*in planta*). It is likely that genes essential for successful infection will be expressed in those structures formed just prior to the invasion of host tissues. Techniques for targeted gene discovery such as amplified fragment length polymorphism mRNA fingerprinting (cDNA-AFLP; Figure 1) and suppression subtractive hybridisation (SSH) have been used to identify genes up-regulated

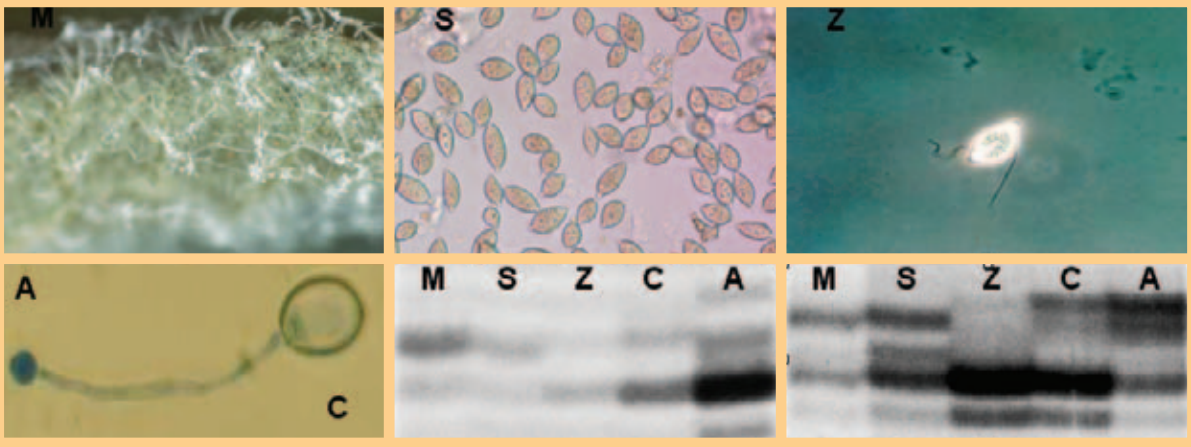

Figure 2 Lifecycle stages in *Phytophthora infestans*. Mycelium (M) forms sporangia (S), that release motile zoospores (Z). The zoospores encyst (C), germinate, and form appressoria (A). Stage specific gene expression can be seen in the sections of cDNA-AFLP profiles (lower right two panels).

in these cell types. Many genes have been identified that can be classified into those required for metabolism, cellular stress, signal transduction, adhesion to host tissue, host cell wall degradation, detoxification and putative virulence factors. Many novel genes, similar to nothing represented in databases, were also discovered. Of particular interest are those genes that encode proteins with predicted signal peptides. These are potentially secreted by *P. infestans* and may interact directly with host plant cells.

Real-time RT-PCR has been used to quantify the relative gene expression profiles for many of the genes identified from cDNA-AFLP and SSH. As expected, all are up-regulated in the cell types from which they were first identified. Many were also shown to be up-regulated to varying levels during the *P. infestans*-potato interaction. For example, glue proteins are expressed at high levels predominantly in those cell types formed just prior to penetration of potato leaf cells. This is in agreement with a hypothesis that some form of sticky matrix is required during the early infection process to prevent the germinated cyst and its appressorium from being dislodged. Of great interest are those genes that showed increasing levels of expression in preinfective cell types, and a high level of expression in the earliest stages of the interaction with potato. In this grouping of genes is a putative secreted small cysteine-rich protein. Genes expressed at these early stages *in planta* are then candidates for influencing the outcome of the interaction. Based on the expression profile for all of the genes discovered to date, they are being prioritized for further functional analyses.

Functional genomics Crucial to understanding the functions of the many genes identified during gene discovery are assays to determine an observable phenotype caused by the presence or absence of specific genes in the interaction. Gene expression vectors based on plant viruses, such as potato virus X (PVX), are being used to express genes predicted to encode proteins with a putative signal peptide; these are known as PEX genes (*Phytophthora* EXported). Infection of potato cultivars containing different resistance genes with PVX expressing PEX genes can reveal if they elicit any response from the potato plants, either in an *R* gene-dependent or *R* gene-independent manner. Plant responses can be observed as either visible symptoms (other than virus infection; Figure 2) or as a specific stimulation of plant defence responses observed through changes in defence gene activation.

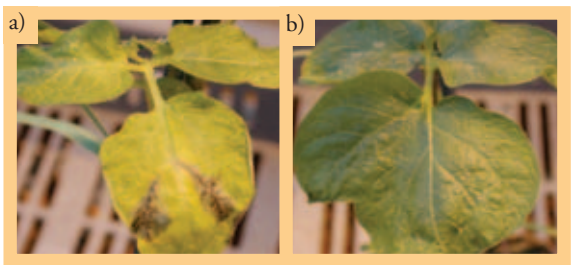

Figure 2 Inoculation of potato with different of *Pex* genes from *P. infestans* cloned into Potato Virus X. a) a defence response has been elicited from the plant. b) No response has been elicited from the plant

Gene silencing has been demonstrated for a small number of *P. infestans* genes through genetic transformation, followed by spontaneous silencing of the introduced copy of the gene. This process, while valuable for determining gene function through its absence, is haphazard in its occurrence and not well suited to the large numbers of candidate genes being generated through any gene discovery program. At SCRI we are piloting a more rapid and transient gene silencing strategy based on the direct introduction of *in vitro* transcribed double stranded RNA (dsRNA) into *P. infestans*. This triggers silencing of the homologous gene in *P. infestans* and is known as RNA interference (RNAi). This technique has been applied to two *P. infestans* genes to date, and initial results have shown a significant gene silencing effect up to 7 days after introduction of the dsRNA. Extended application of RNAi to *P. infestans* functional genomics has the potential to accelerate the determination of gene function.

Future prospects Gene discovery, either targeted (SSH) or not (ESTs), has provided a strong platform for the identification of genes involved in the *P. infestans*-potato interaction. Further strategies for *P. infestans* gene discovery, including microarray analysis of over 15 000 *P. infestans* genes, will add significantly more candidates to the growing list of those for which a function in the interaction must be determined. Improved functional genomics tools, although not as high throughput as gene discovery, are now in place for *P. infestans*. The application of these tools will aid in the identification of genes required for *P. infestans* to be a successful pathogen of potato. Studies of these mechanisms are vital as a counterpart to genomics studies of the host plant potato and its response to infection. Information and insights from both sides of the interaction will better inform future late blight management strategies.