## Late blight in the 21st century: new tools for an old problem

S.C. Whisson, A.O. Avrova, M. Armstrong, E. Campbell & P.R.J. Birch

ver 150 years have elapsed since the first epidemics of a plant disease called late blight devastated the potato crops of North Western Europe. The severe impact of these epidemics on communities that relied heavily on potato as a staple food provided a major impetus to the study of plant pathology. Late blight disease was shown to be caused by a 'fungus' called *Phytophthora* (plant destroyer) *infestans* and since these early epidemics of late blight, intense efforts have been directed to identify new ways to control the disease. Potato cultivars containing major gene resistance (single resistance genes conditioning complete resistance) were used initially to control late blight, but P. infestans rapidly overcame the deployed resistance genes. Use of fungicides has also had some success (metalaxyl for example), but P. infestans can adapt to become resistant to them. In addition, agrochemicals require many repeat sprayings throughout the growing season, sometimes as often as every 5 to 7 days, to be effective. The cost of chemical treatments can greatly diminish the monetary returns from a potato crop, and spraying of chemicals is not viewed by many to be environmentally friendly.

Currently, late-blight is considered the most serious constraint to the production of potato, the world's fourth most valuable crop plant. Worldwide losses due to late blight and control measures are estimated to exceed £3 billion annually. *P. infestans* is thus regarded as a threat to global food security. Despite its economic importance, relatively little is known about the fundamental molecular biology underlying *P. infestans* development, its capacity to cause late blight (pathogenicity), and the factors that restrict late blight to potato and tomato (host-specificity and avirulence). Modern molecular and biochemical studies have placed *P. infestans* in the oomycetes, a class of organisms distinct from the true fungi, and more closely allied to heterokont algae. The oomycetes contain many other important plant pathogens such as *P. sojae* (soybean root rot), *Peronospora parasitica* (Arabidopsis downy mildew), *Bremia lactucae* (lettuce downy mildew), and *P. ramorum* (sudden oak death).

> In recent years, research efforts directed at P. infestans have begun to utilise the latest genomics technologies to further the knowledge of this plant pathogen. At SCRI there is an integrated program of genomics research targeted at understanding the molecular mechanisms involved in the P. infestans/potato interaction. As a result, P. infestans is fast becoming the model oomycete plant pathogen for conducting such studies, as resources such as expressed sequence tags (ESTs), bacterial artificial chromosome (BAC) libraries, and genetic linkage maps have been developed. In addition, techniques for targeted gene discovery, transformation for analysing gene function, and gene silencing have also been greatly improved (reviewed in

Birch and Whisson, 2001). We aim to use all the resources and techniques available for studying *P. infestans* to better understand the development of late blight, and exploit this knowledge for more durable control measures in future.

**Life cycle stage specific gene expression** The life cycle of *P. infestans* is complex, involving differentiation into as many as eleven different cell types. These cell types are highly specialised for life cycle stages involved in sexual and asexual reproduction, propag-

ule dispersal, spore germination, host penetration, and biotrophic and necrotrophic phases of infection. The differentiation of so many cell types requires the upor down-regulation of many genes. Of particular interest are those cells formed shortly before and early in the interaction with the host plant, such as germinated zoospore cysts, germ tube, appressorium, infection peg, and infection vesicle. *P. infestans* is hemi-biotrophic and can form most of these cell types in the absence of a host plant. We anticipate that many genes required for successful infection of the host plant will be up-regulated in these stages. Indeed, preliminary results indicate that some of the genes isolated to date are up-regulated in these cell types and also *in planta*.

Crucial to investigating and exploiting natural resistance mechanisms to *P. infestans* is an understanding of the molecular events occurring during early interactions between *P. infestans* and plants. This stage of the interaction of the pathogen elicits plant defences (Birch *et al.*, 1999). The target elicitors are critical signalling molecules whose identity is central to understanding the outcome of the interaction.

Suppression subtractive hybridisation (SSH) and amplified fragment length polymorphism (AFLP)based mRNA fingerprinting (cDNA-AFLP) have been used to identify genes up- and down-regulated during specific stages of the life cycle and infection. These powerful, PCR-based techniques are routinely used at SCRI for discovering genes in a range of pathogen/plant interactions (see articles by Blok et al. and Toth et al.). SSH cDNA has also been labelled as a complex probe and used to screen a BAC library generated at SCRI that contains the P. infestans genome arrayed on filters (Whisson et al., 2001). In this approach, BAC clones containing clusters of coregulated genes were identified. Full-length genes, derived from both cDNA-AFLP and SSH, can be determined from corresponding BAC clones, or from EST databases.

From both cDNA-AFLP and SSH of pre-infective stages, we have identified many *P. infestans* genes that are up-regulated during the infection process. Many of these show significant sequence identity to pathogenicity factors such as cell wall degrading enzymes, but many are more closely associated with stress and defence responses. This suggests a hostpathogen interaction where both pathogen and host are simultaneously attacking and defending. A more

targeted gene discovery approach focussing on the two major phases of infection, biotrophic and necrotrophic, has resulted in the discovery of two novel gene families in *P. infestans* that are very tightly regulated during infection. One of these has been shown to be highly up-regulated at the infection time point corresponding to the transition phase from biotrophy (parasitism) to necrotrophy (cell killing). Technical advances in the detection of gene expression using real-time RT-PCR technology has allowed us to identify and quantify levels of gene expression in P. infestans/potato interactions. This is particularly important at the very early stages of infection, where the fate of the interaction (resistance or susceptibility) is decided. This is the first report quantifying pathogen gene expression at this early infection stage in any plant/pathogen interaction.

Avirulence genes *P. infestans* has a narrow host-range and a hemi-biotrophic mode of infection. Host species and race specificities are determined soon after penetration of the host plant (12 - 24 hours) and are reliant on complex signalling between host and pathogen, and within the pathogen itself. Resistance (whether mediated by the products of resistance [*R*] genes in the host, or as yet uncharacterised receptors in the non-host) is based, primarily, on recognition of a particular elicitor component (or avirulence factor) from the pathogen. Recognition of pathogen avirulence factors triggers the hypersensitive response (HR), a form of localised programmed cell death that restricts further spread of the pathogen, in both resistant host and non-host plants

At least 11 R genes active against races of P. infestans have been introgressed from Solanum demissum into the cultivated potato, S. tuberosum. As yet, only one potato R gene (R1) responsible for race specific recognition of P. infestans has been isolated. However, the positions of R1, R3, R2, R6, and R7 have been genetically mapped. Only a few avirulence genes have been cloned from fungi (e.g. from Cladosporium and Magnaporthe) and one from the oomycetes. In the biotrophic fungi, which often form structures called haustoria that exist within the living plant cell, nothing is known about the mechanisms of delivery of potential pathogenicity or avirulence gene products.

We have adopted two approaches to identify avirulence genes in *P. infestans*. Firstly, we are using a map based (or positional) approach to target avirulence gene Avr2 (matching the resistance gene R2). Here we are characterising the genetics of avirulence to pin-



## Integrated physical, transcriptional and comparative mapping: framework

**Figure 1** Overview of the SCRI *Phytophthora infestans* integrated genomics programme. Genetic and physical maps will yield insights into overall genome architecture. A transcriptional (gene) map, coupled with transcriptional profiling (gene discovery) will yield information on genes expressed at specific life cycle stages, or during infection of potato. Comparisons with other oomycete pathosystems are possible through a comparative genomics approach. The example given is for a hypothetical avirulence locus (*Avr*), located in a cluster of genes that are up-regulated during infection of potato; a similar locus would also exist in a comparative system (called *AvrPs*).

point the genome region containing *Avr2*. We will then use this genetic knowledge as a basis for identifying the gene from our BAC library.

A second approach is using association genetics. From a large set of P. infestans ESTs we, in collaboration with S. Kamoun (Ohio State University, USA), have identified a small selection of P. infestans genes that will be assayed for single nucleotide polymorphisms (SNPs) associated with avirulence towards specific potato R genes. These genes were selected on the basis of similar features to avirulence genes cloned from other plant pathogens, and the presence of a predicted signal peptide for secretion. Since the avirulence molecules themselves must cross the plasma membrane of the pathogen to be perceived by the host plant, avirulence gene sequences are predicted to encode a signal peptide for secretion. This has been a highly rewarding exercise, with our first screen of sequences identifying a SNP completely associated with Avr3 (matching R3). Further experimental work is now required to characterise the function of this candidate Avr gene.

**Structural genomics** Similar to the project aimed at reconstructing the genome of the potato pathogen *Erwinia carotovora* (see article by I. Toth *et al.*), efforts are underway at SCRI to understand the organisation of the *P. infestans* genome. We aim to link genetic, physical and transcriptional maps for *P. infestans* to allow rapid positional cloning of genes involved in avirulence, and to aid identification of genes involved in sexual compatibility and resistance to agrochemicals.

A genetic map, consisting of AFLP markers and avirulence loci, has been constructed. The SCRI *P. infestans* BAC library was based on an  $F_1$  individual from the same cross used to construct the genetic linkage map (Whisson *et al.* 2001). Mapped AFLP markers are being used to anchor the physical map by AFLP fingerprinting pools of the BAC clones, and non-polymorphic amplified fragments will be used to extend the physical map. A physical map of the *P. infestans* genome will serve as a central resource for the international *P. infestans* research community to locate biologically derived genomic data such as ESTs, and anchor any future genome sequencing for *P. infestans* (Figure 1). By locating ESTs on BAC clones, generich regions of the genome will be revealed. It will also be possible to assess if co-location of co-regulated or related genes occurs in *P. infestans*, as well as any gene duplication. When the transcriptional map is combined with a genomic physical map, a broader view of genome organisation will also emerge.

Integration of the published genetic linkage map with a physical map of contiguous BAC clones is underway and is expected to cover a large proportion of the *P. infestans* genome. Many ESTs from Phytophthora sequence databases, and from the SCRI gene discovery program, have also been placed on BAC clones. Initial results indicate that genes specifically regulated during pathogenesis on potato are clustered in the *P. infestans* genome.

**Comparative genomics** *P. infestans* is a hemibiotrophic oomycete pathogen that predominantly infects the foliage of potato plants. Related oomycete pathogens such as P. sojae and Peronospora parasitica have very different life styles to P. infestans. P. sojae is a hemi-biotrophic pathogen of soybean that infects predominantly through the roots, and *Pe. parasitica* is a strictly biotrophic pathogen that infects Arabidopsis thaliana foliage. Our gene discovery programme also includes comparative analyses with the pathogens mentioned above, especially Pe. parasitica. Comparisons of genomic organisation for similar genes in the P. infestans genome with other oomycete genomes will reveal common or distinct regions that may include genes involved, respectively, in pathogenicity and host-specificity. Conservation of genome organisation in these regions may suggest a central role in host infection.

A comparison between genomes involving a series of genes linked to the *Atr*1Nd avirulence locus in *Pe. parasitica* (Rehmany *et al.*, 2002) has shown that the order of genes in *P. infestans* is conserved. However, we do not know if this locus is also involved in avirulence in *P. infestans*. Other genome comparisons between these pathogens are ongoing.

**Summary** Diseases of crop plants have a significant impact on the economics of agriculture in the UK and many other countries. Current late blight control measures relying solely on major resistance genes in potato are not effective, and spraying with chemicals is expensive and increasingly less socially acceptable. A greater understanding of plant resistance and its underlying biochemical mechanisms will be of use in the development of durable resistance in potato (see article by V. Blok *et al.*). However, conducting an integrated programme of genomics research in parallel on the pathogen will yield those components of the interaction that must also be considered in any future resistance deployment.

Implementation of this integrated research programme at SCRI, studying both sides of the interaction between host and pathogen will lead to an intimate knowledge of the mechanisms and processes involved in pathogenicity, avirulence and resistance, and pathogen life cycle. Identification of biochemical or signalling pathways involved in infection and recognition will be important in designing strategies or targeting chemicals that allow broad-range control of these important pathogens. Exploitation of this knowledge will enable novel control strategies for late blight to protect potato production in Scotland, and many other potato producing countries worldwide.

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