Host pathogen interactions and crop protection

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The resistance of certain plants to bacterial and fungal diseases can be pre-formed, as anti-microbial compounds stored in healthy tissue, or as impenetrable barriers of dead or modified cells. However, much more important is resistance in which the host reacts to infection and metabolic changes create an environment within the plant that is unsuitable for the pathogen. Such active resistance is initiated by host recognition of attack by the pathogen. Central to host-pathogen recognition, especially with biotrophs, is the gene-for-gene concept which remains largely intact despite many years of argument between proponents of vertical and horizontal resistance. In any case, the distinction between the two has become slightly blurred as horizontal resistance in a number of plants has been mapped to major quantitative trait loci (QTLs) and may be at most oligogenic rather than polygenic. Moreover, both forms of resistance often involve activation of many of the same metabolic pathways within the plant, e.g. *the synthesis of pathogenesis-related proteins and phytoalexins.*

The increasing characterisation of sequences of resistance genes from the host, and virulence genes from the pathogen and their products, will result, hopefully, in an improved understanding of how the recognition mechanism initiates the cascade of events which eventually is manifest as a resistant reaction. However, although many genes and metabolites from the subsequent cascade have been characterised, many others remain undiscovered. A new research project (RO 494) designed to isolate such genes from potato infected by *Phytophthora infestans* (late blight) and *Erwinia carotovora atroseptica* (blackleg), was initiated in 1997. Already, using cDNA-AFLPs and new PCR-based cDNA subtraction techniques, a number of genes not previously reported from plants have been isolated. Some of these, *e.g.* serine palmitoyltransferase, which is implicated in signalling the onset of programmed cell death or apoptosis in mammalian cells, are only up-regulated in the earliest stages of an hypersensitive response to infection with *P. infestans*. In parallel work, the same techniques are being used to isolate genes specifically activated within a pathogen *e.g.* genes produced by *P. fragariae* var. *fragariae* but not by the closely related var. *rubi.*

Activation of genes in diseased potatoes results in the production of a whole battery of proteins and other

compounds not found in healthy plants or present in much smaller amounts. Among them are phytoalexins, low molecular weight anti-microbial compounds implicated in slowing or halting the progress of pathogens through host tissue. Dr Gary Lyon presents his unparalleled knowledge of this area of potato metabolism in an article elsewhere in this report (see p.118). Uniquely, he has distilled everything into a comprehensive metabolic map or poster which is freely available on the World Wide Web.

Elsewhere within FBPP, the effort on molecular diagnostics continues. The lessons learned from the highly successful HDC-supported programme on *Phytophthora* diagnostics are now being applied practically in an SCRI-led programme under the European Union's 'Standards, Measurements and Testing' programme. The aim is to develop to ISO standards, PCR-based tests for the presence of *P. fragariae* (red core) in commercial strawberry propagation stocks throughout Europe. Similar diagnostics for *Spongospora* on potato tubers, funded by BPC, have proven very sensitive and early results suggest that they could be applied to soil as well as plants. Recently, the work was extended to other blemish pathogens, in particular, to black dot (*Colletotrichum coccodes*), silver scurf (*Helminthosporium solani*) and common scab (*Streptomyces* spp.), as part of an open contract with MAFF. Similar progress has been made with bacterial diseases, with PCR-based tests for detecting *Erwinia* on potatoes and *Xanthomonas* spp. attacking beans.

All of this work, which is funded by contract, is integrated with ongoing basic core research. For example, the development of *Phytophthora* diagnostics has led to molecular characterisation of a very comprehensive range of *Phytophthora* species in joint work with Professor Brasier of the Forestry Commission. Nearly every available *Phytophthora* species has been included. Likewise, PCR detection of *Erwinia* is closely linked to core work on molecular diversity within that genus. New studies on the molecular diversity present in cereal rusts hopefully will yield similar tangible results.

Research on various resistance strategies, as well as mechanisms of resistance, continues to be a major focus within the Virology Department, where both the mechanisms of natural resistance to plant viruses as well as pathogen-derived resistance to plant viruses are being examined.

A resistance strategy against groundnut rosette virus (GRV) was demonstrated in *Nicotiana benthamiana*, utilising a hypovirulent satellite RNA as a transgene to suppress the replication of GRV. In fact, two types of resistance were observed. In one, GRV, as well as a pathogenic satellite RNA, were suppressed by the transgenic hypovirulent satellite RNA. In the other, the pathogenic satellite RNA was suppressed, but GRV was not, leading to a reduction in symptom expression i.e. tolerance. With improvements in transformation technology, it is hoped that such resistance could be introduced into groundnut (*Arachis hypogaea*) in sub-Saharan Africa, where groundnut rosette disease is a severe agricultural problem.

Pathogen-derived resistance to potato mop-top virus (PMTV) is being tested in potato, where there is no natural resistance to this virus. PMTV is an important potato pathogen in Scandinavia, and is becoming increasingly important in China. The mechanism of this resistance has been examined in transgenic *N. benthamiana* and involves the presence of the virus coat protein, expressed from the transgene, rather than transgene-mediated silencing mechanisms mediated by RNA. The resistance was maintained against a number of Scandinavian strains of PMTV.

Natural resistance to potato virus Y (PVY) and potato virus A (PVA) genes has been analysed. The Ry_{sto} gene from *Solanum stoloniferum* confers resistance to PVY, PVA and PVV, while the *Ra* gene, also from *S. stoloniferum*, confers resistance to PVA alone. The Ry_{sto} gene has been mapped and is being isolated elsewhere. The *Ra* gene is being mapped to facilitate its isolation, and potato protoplasts are being used to compare the mechanisms of resistance to PVY and PVA.

The mechanism of replicase-mediated resistance to cucumber mosaic virus (CMV) was analysed further. A hypothesis stating that the movement protein (MP) was inhibited from either binding to or moving through plasmodesmata, was analysed using two approaches: (1) The CMV MP, fused to the green fluorescent protein (GFP), was expressed from the potato virus X vector and was shown to associate with plasmodesmata; (2) the same MP-GFP fusion was expressed from CMV RNAs with limited cell movement, and the MP-GFP was shown to move through plasmodesmata. These observations refute the hypothesis that replicase-mediated resistance inhibits virus movement by inhibiting the MP from either associating with or passing through plasmodesmata.

A novel form of resistance to CMV was observed in transgenic tobacco expressing CMV RNA 1. While

some regenerated plants could complement the replication of CMV, when only RNAs 2 and 3 were added, the selfed progeny of some of these plants showed resistance to systemic infection of inoculated CMV, even though they were still able to complement replication in the inoculated leaves. The mechanism of resistance involves a sequence-specific inhibition of RNA 1 accumulation, but does not involve either constitutive or inducible gene silencing. Further work on this mechanism and the other mechanisms of virus resistance described above, will increase our ability to apply different resistance strategies and prevent infection of agriculturally important plants by viruses.

The widespread and variable virulence of the white potato cyst nematode (PCN, *Globodera pallida*) is hampering progress in breeding for resistance. Consequently, attaining a better understanding of virulence differences in PCN has been a priority. Molecular approaches have been applied to explore the proposition that, as PCN is an introduced pest, virulence differences between populations derive from differences in the gene pools introduced. Exceptionally virulent populations, such as Luffness from Scotland, were of particular interest, and RAPD analysis supported the view that it represents a distinct introduction. Avirulent pathotype Pa1 populations were also confirmed as being genetically distinct and therefore probably deriving from a different introduction to the majority of populations which are of intermediate to low virulence.

As *G. pallida* is now widely distributed in the UK and Europe, it seems likely that post-founder events will also have influenced current patterns of virulence. This was supported by a molecular genetic analysis. Pathotypes Pa2 and Pa3 could not be distinguished and were shown to represent the extremes of a continuum of biological and genetic variation. Analysis with inbred lines demonstrated that this range of variation could be produced by genetic drift. It was also demonstrated that *G. pallida* is extremely heterogeneous, and that it readily responds to selection, even by apparently susceptible cultivars of potato. Resistant cultivars produced even greater selection.

Analysis of the internal transcribed spaces (ITS) region of ribosomal DNA (rDNA) provided evidence for substantial gene flow within UK populations. Restriction enzyme digestion of the amplification product from the ITS region with RsaI showed that Pathotype Pa2/3 populations contained three distinct ribo-types (Fig.1). One of these was found in Pa1 and another in a population from South America (P5A), each of which contained a single ribo-type. The third ribo-type was present in a population (Pa375) from the Netherlands which also contained the ribo-type found in Pa1.

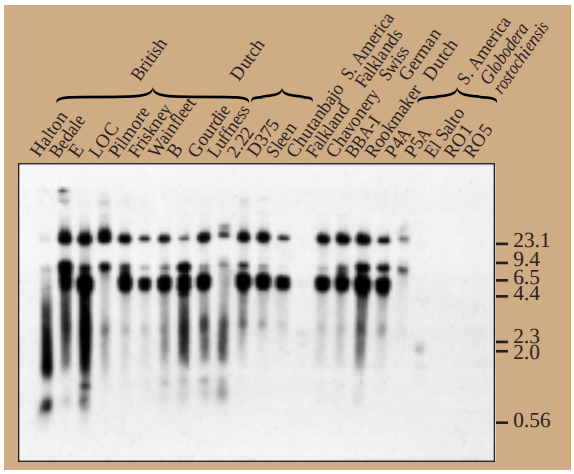

Figure 1 RsaI digestion products of ribosomal PCR products from 17 *G. pallida* populations. Sizes of digestion products based on sequence information are indicated.

Analyses of mtDNA have revealed even greater divergence in *G. pallida*. Ten mtDNA probes, derived from a Pa2/3 population library were used to probe the DNA of populations of *G. pallida* from the UK, Europe and South America. Most, or all of the probes bound to total DNA from most of the UK and European populations, and to one population from South America (El Salto). However, less hybridisation was observed with the Luffness and Pa1 populations and several probes failed to hybridise to the P5A population (Fig.2).

The unique structure of the mtDNA genome of *G. pallida* was the greatest surprise. It was shown to be

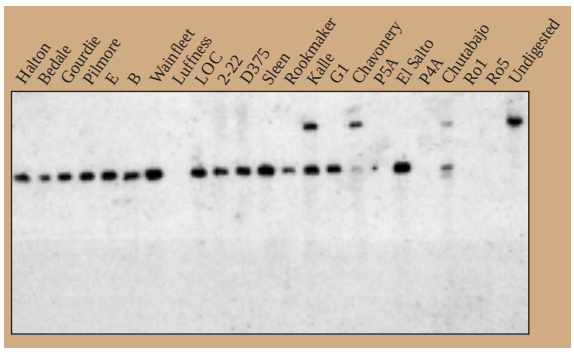

Figure 2 RsaI digestion products of ribosomal PCR products from 17 *G. pallida* populations. Sizes of digestion products based on sequence information are indicated.

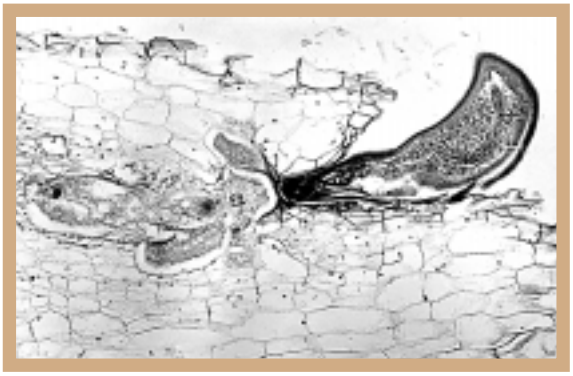

Figure 3 Young female cyst nematode (right) with enlarged cells forming the syncytium on which it depends for its food (left).

comprised of a population of small, circular DNAs that individually are too small (<13kb) to contain all the genes required for a fully functional mitochondrial genome. Various types of multi-partite mtDNA genomes are found in plants, fungi and some protozoa, but have never before been reported from a metazoan. Such an arrangement poses many questions, including how and why it has arisen. This may relate to the unusual biology of PCN, which includes a capacity to change sex, depending on the environment, and to persist as unhatched eggs for >20 years between potato crops. Current research is focused on sequencing *G. pallida* mtDNA.

Research continues to isolate the *Hero* gene from tomato, which we showed conferred c. 80% resistance to *G. pallida* Pa2/3. Cosmids spanning the *Hero* locus have been supplied by colleagues in Germany. Complementation studies seeking to transform potato with such cosmids are in progress. We are also involved in the early stages of the map-based cloning of genes from potato with quantitative resistance but, as avirulent nematodes are the probes, such research requires access to populations of *G. pallida* with specific avirulence characteristics.

Cyst nematodes are endoparasitic, sedentary and biotrophic root pathogens. To become adult, they have to induce the root cells at their permanent feeding sites to become enlarged and multi-nucleate, thereby providing the developing juvenile with a rich supply of food. As these enlarged "syncytia" (Fig.3) have to remain alive and metabolically active for the whole of the nematode's life cycle, it is imperative for the nematode that it induces a susceptible rather than a resistant response. Whether an incompatible or compatible response results, depends on the reaction of the host to the secretions produced/injected by the nematode. We have developed techniques of collecting nematode secretions in sufficient quantities to analyse and to use for antibody production. We are also screening cDNA libraries made from PCN invasive stage juveniles in an attempt to isolate genes encoding secreted molecules.

Direct biochemical analysis of secretions has revealed the presence of a range of biologically-active molecules including antioxidant enzymes, plant cell wall-degrading enzymes and metalloproteases. Such biochemical studies are still extremely challenging due to the enormous numbers of nematodes required to produce sufficient secretions for even the most basic of biochemical studies. More progress has been made in characterising genes isolated from cDNA library screening. These genes can be sub-cloned into expression vectors which allow the production of almost unlimited quantities of protein for biochemical analysis, thus enabling details of the functional roles of each protein to be investigated. Genes investigated in this way include GPSEC-2, a secreted fatty acid binding protein from *G. pallida*, and a thioredoxin peroxidase from *G. rostochiensis.*

GPSEC-2 was isolated from a *G. pallida* expression library using an antiserum which bound to the surface of invasive stage juveniles of this nematode. Sequence analysis showed it to be extremely similar to proteins produced by a range of free-living and animal parasitic nematodes. Functional studies undertaken on protein produced from this gene showed that, like its counterparts in animal parasites, GPSEC-2 was capable of binding a wide range of fatty acid ligands including linolenic and linoleic acids. These fatty acids are used as substrates by plant lipoxygenases in order to generate free radicals; this reaction forms one of the first lines of defence used by plants against attack by pathogens. Subsequent work has shown that GPSEC-2 is capable of inhibiting the activity of this enzyme *in vitro* suggesting that it may have a secondary role in protecting the pathogen from host defence responses.

Work on the second species of PCN, *G. rostochiensis*, led to the isolation of a gene encoding a thioredoxin peroxidase. This gene was isolated from a cDNA library using an antiserum raised against secretions collected from this nematode. Biochemical studies, in collaboration with the University of Dundee, on the protein produced from this gene showed that, like its counterparts from animal parasitic nematodes, it operates in a different pathway to thioredoxin peroxidases from other organisms, making it an excellent candidate for targeting with novel control methods.