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Isolating Genes Involved in Pathogenicity of Wheat Brown Rust *Puccinia recondita*

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ABSTRACT. *Puccinia recondita f. sp. tritici is the causal agent of brown rust of wheat and occurs throughout wheat growing countries. Because of the obligate biotrophic nature of P. recondita it cannot be studied in vitro. The traditional molecular approach to investigate such a plant pathogen system would be to make a cDNA library from infected plant material and differentially screen this with cDNAs prepared from either the individual pathogen or host. A new approach to the search for pathogenicity-related genes in P. recondita is being taken using the technique of "Differential Display". cDNA has been made from the total mRNA population existing in a brown rust infected wheat leaf at 24 hours post inoculation and from uninfected wheat leaves of the same age. Subsets of these populations have been PCR amplified, the products separated on denaturing polyacrylamide gels, and visualised by autoradiography. PCR products which appear at higher levels in the infected wheat sample indicate genes which may be up-regulated in either the host plant or the pathogenic fungus during infection. Products at reduced levels in the infected sample correspond to genes which may have been down-regulated during the infection process. Examples of each product type have been isolated. These DNA fragments may contain part of the sequence of a pathogenicity-related gene and the source of these genes is being determined by individual hybridisation with genomic DNA from the wheat and from P. recondita. Full-length cDNAs are being isolated from a cDNA library made from wheat infected with brown rust.*

INTRODUCTION

Puccinia recondita f. sp. tritici is the causal agent of brown rust of wheat and occurs throughout the world-wide distribution of wheat. Control of

this complex biotrophic pathogen depends mainly on the use of resistant wheat cultivars. The identification of genes involved in the pathogenicity of *P. recondita* could provide useful information to wheat breeders and aid the development of novel control methods for brown rust.

Although some rust fungi can now be cultured axenically (Fasters *et al.*, 1993) *P. recondita* cannot yet be studied *in vitro*. The traditional molecular approach used to investigate such a plant pathogen system would be to make a cDNA library from infected plant material and differentially screen this with cDNAs prepared from either the individual pathogen or host. A new approach to the search for pathogenicity related genes in *P. recondita* is being taken using the technique of differential display (Liang and Pardee, 1992; Liang *et al.*, 1995).

The aim of this research project is to identify genes which are expressed during compatible interactions of brown rust with wheat using the techniques of differential display and cDNA library screening.

MATERIALS AND METHODS

Eight day old seedlings of a susceptible wheat variety (*Triticum aestivum* cv. alexandria) were inoculated with wheat brown rust by dusting the plants with a rust spore/talc mixture. The plants were incubated in high humidity (allowing free water to be present on leaf surfaces) for 24 hours then sprayed with water to remove talc and spores. The first true leaves were harvested and immediately frozen in liquid nitrogen.

Total RNA was extracted from inoculated plants and from a set of uninoculated control plants by grinding the tissue in liquid nitrogen followed by buffered phenol-chloroform extraction of proteins and carbohydrates, removal of DNA by NaAc precipitation and precipitation of the isolated RNA using LiCl. After initial extraction the RNA was treated with RNAase-free DNAase to remove any contaminating DNA (Liang *et al.*, 1995). The purity of the RNA was checked by gel electrophoresis and by spectrophotometry (Sambrook *et al.*, 1989).

cDNA was made from the purified RNA using reverse transcriptase and 8 different 14mer anchor primers designed to amplify from the whole of the mRNA population (Liang *et al.*, 1995).

Subsequent PCR (polymerase chain reaction) of the cDNA fragments was carried out using the appropriate anchor primer plus an arbitrary 10mer primer and ^{35}S labelled NTPs (nucleotide triphosphates) (Liang *et al.*, 1995). The resulting products were separated on denaturing acrylamide gels and visualised by autoradiography.

RESULTS AND DISCUSSION

PCR products which appear at higher levels in the infected wheat sample indicate genes which may be up-regulated in either the host plant or the pathogenic fungus during infection. Products with reduced levels in the infected sample correspond to genes which may have been down-regulated during the infection process.

So far 8 anchor primers have been tested in all combinations with 5 arbitrary primers and 26 bands have been found which indicate differential mRNA expression on comparison of the labelled PCR products from inoculated and uninoculated wheat. These bands have been cut from the gels and the DNA reamplified and then cloned.

Previous studies have shown that when a band is cut from the gel, reamplified and cloned it is usually shown to have consisted of 2 or more DNA species which have co-segregated during electrophoresis (Liu and Raghothama, 1996). It has therefore been necessary to choose approximately 6 clones for each band studied and check whether or not the inserts show the expected expression pattern using Northern analysis.

Northern analysis of several clones is underway and these will be used to screen a wheat/wheat brown rust cDNA library.

CONCLUSIONS

Using differential display DNA probes have been generated for use in screening a cDNA library for wheat brown rust pathogenicity-related genes.

Full length clones isolated from the library will be sequenced for comparison with known pathogenicity genes from other pathogen-host systems.

Studying the expression of pathogenicity-related genes over time and stages of disease development could help to elucidate the mechanisms of pathogenicity in *P.recondita*.

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