Analysis of pathogenicity factors in the *Pyrenophora graminea*-barley interaction

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Aim

Isolation and study of pathogenicity genes of *P. graminea* with different strategies:

- AFLP-TP analysis of differential expressed genes during *P. graminea* infection of barley
- Homology-based reverse genetics
- Characterization of *PIG14*

Introduction

The study of the interaction between barley and *Pyrenophora graminea* is a model for some seed-borne diseases. Major results achieved in previous research were:

- a transformation system for *P. graminea* has been used for REMI analysis and to obtain GUS + isolates which were employed in histological studies of the infection process
- isolation and characterization of the Pg toxin produced by the fungus
- isolation of a new fungal gene (*PIG14*) involved in pathogenesis of *P. graminea*, by means of RT-PCR differential display

AFLP-TP analysis of differential expressed genes

An improved cDNA-AFLP approach (AFLP-TP) was used for the analysis of gene expression during infection. The main features of this technique are: 1) **high sensitivity**; 2) **unique fragment for each cDNA**; 3) **proportionality between band intensity and relative amount of different cDNA**; 4) **lower number of selective amplifications needed (32 instead of 256)**

- RNA was extracted from artificially infected barley embryos (cv Thibaut, resistant and cv Mirco, susceptible), sampled at 7, 10, 15 days post inoculation (dpi)
- seven out of the 32 primer combination tested until now yielded a total of 300 differentially expressed bands (ca. 5% of the total) (Fig.1). Each differentially expressed band is amplified and cloned into a T-vector. An example of up- and down-regulated cDNA fragments is shown in Fig. 2
- *P. graminea* differential expressed bands will be sequenced and aligned to known sequences present in GenBank

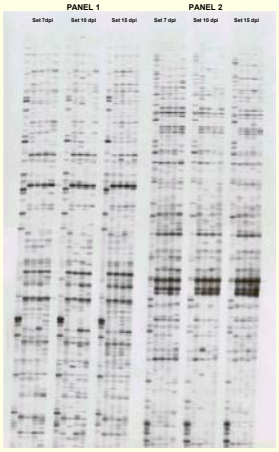


Fig. 1 - Autoradiography of typical AFLP-TP gel. Each panel represents an arbitrary primer combination. For each set (from left): fungus, resistant inoculated, resistant control, susceptible inoculated, susceptible control.

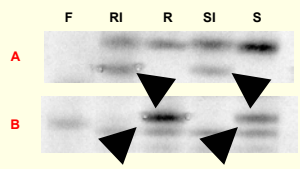
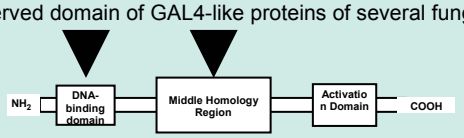


Fig. 2 - Examples of up-regulated (A) and down-regulated (B) cDNA fragments upon infection of resistant and susceptible barley embryos with *P. graminea*. (F: fungus; RI: resistant inoculated; R: resistant control; SI: susceptible inoculated; S: susceptible control).

Homology-based reverse genetics

The presence and the function of a gene coding for a transcriptional activator of the fungal zinc cluster ($Zn[II]_2Cys_6$) family (GAL4-like) (Dufresne *et al.*, 2000. The Plant Cell, 12, 1579-1589), is currently under study in *P. graminea*.

Degenerate primers have been designed on the basis of conserved domain of GAL4-like proteins of several fungi.



PCR reactions are in progress in order to:

- check the presence of the gene in *P. graminea* genomic DNA. If present:
- study the involvement of the gene in the biotrophic/necrotrophic switch of the fungus during pathogenesis.

Characterization of *PIG14*

PCR analysis with *PIG14* specific primers, conducted on genomic DNA of several pathogens, showed that the expected band of 440 bp was:

- present in 23 isolates of *P. graminea* with different degree of virulence, 5 of *P. teres* f.s. *maculata* (Pt-SPOT), 5 of *P. teres* f.s. *teres* (Pt-NET) and one each of *P. japonica* and *P. avenae*
- absent in *Cochliobolus sativus, C. miyabeanus, Pyrenochaeta lycopersici* and *Diaporthe phaseolorum*
- disruption analyses are needed to understand the function of the gene

Sequence analysis showed that *PIG14* is very conserved also in other fungal species. The unique silent nucleotide substitution allowed the discrimination between isolates of the two *formae speciales* of *P. teres* tested, by PCR – Sau3A digestion

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