**PR7.8**

**Genome mining and functional genomics of small secreted proteins (SSPs) in *Cladosporium fulvum, Mycosphaerella graminicola* and *M. fijiensis***

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Fungal pathogens secrete effector proteins to target and manipulate host plants for successful infection. They primarily function as virulence factors but during co-evolution plants have evolved resistance proteins to recognize them resulting in effector-triggered immunity (ETI). Successful pathogens, in turn, evolved additional effectors to evade recognition or to suppress ETI resulting in host susceptibility. In this study we mined the genomes of *Cladosporium fulvum, Mycosphaerella graminicola* and *M. fijiensis* and identified 289, 266 and 180 SSPs, respectively, that represent a common feature of effector proteins (<300 aa residues and containing ≥4 cysteine residues). We used a combined proteomics and expression profile approach and selected over 100 SSPs for further functional analysis. The SSPs are cloned into an *Agrobacterium tumefaciens* overexpression vector with or without the PR-1A signal peptide sequence for transient expression *in planta*. We are testing the effect(s) of SSPs in suppression of HR or necrosis induced by Inf1, CfHNNI1, BAX or Cfs/Avr gene pairs in tobacco plants. The role of some promising candidates in virulence will be assayed by gene knock-out studies. In addition, we will produce *M. graminicola* SSPs in an *Escherichia coli* heterologous expression system and purify them using FLAG affinity purification. Subsequently, *M. graminicola* SSPs will be infiltrated in a set of differential cultivars to identify SSPs that are able to cause HR or necrosis. Systemic overexpression of *M. graminicola* SSPs will be performed using the BSMV-mediated expression system in wheat differentials. Preliminary data and comparison of SSPs from the different fungal pathogens will be discussed.

*Rahim Mehrabi is financially supported by an ERA-PG grant (ERA-PG 31855.004) and Pierre JGM de Wit by the Royal Netherlands Academy of Arts and Sciences.*

**PR7.9**

**Characterisation of two necrosis and ethylene inducing protein-like (Nlp) genes from *Sclerotinia sclerotiorum***

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The necrotrophic phytopathogen, *Sclerotinia sclerotiorum,* induces plant cell death in order to colonise host plants and release nutrients. This pathogen is known to secrete various compounds, including oxalic acid and lytic enzymes during infection. Infection is often facilitated in other necrotrophs (such as *Fusarium oxysporum* and *Botrytis cinerea*) by the secretion of small, phytotoxic effector molecules (including necrosis and ethylene inducing peptides, NEPs). We have cloned two genes from *S. sclerotiorum* with significant similarity to *NEP*s called *NEP-like1* (*Nlp1*) and *Nlp2*. Both NLPs appear to induce necrosis when transiently expressed in *Nicotiana tobaccum* and *N. benthamiana*. Multiple 35S fusion constructs, designed to investigate the effect of *in planta* NLP protein expression (with or without signal peptides) have also been completed and transformations are currently underway. Both genes have also been expressed in *Pichia pastoris* resulting in purified NLP1 and NLP2 that will be used for further transient assays to assess the specific response induced in host plants when presented with one or both proteins. Multiple Nlp1RNAi mutant lines have been generated which display inhibited growth rates both *in vitro* and *in planta*; further characterisation of these will be presented. Expression localisation studies are also underway using *NLP* promoter:GFP fusion constructs in transformed *S. sclerotiorum*.