agropolis fondation

Year of CfP: 2008

Project No 0802-023

Project title: Development of a system for high throughput functional analysis of *Magnaporthe grisea* virulence effector proteins

Unit managing the project: BGPI (Biology and genetics of plant/pathogen interactions) (CIRAD, INRA, Montpellier SupAgro)

Project leader: Thomas Kroj (kroj(a)supagro.inra.fr)

Country involved in the project: China

Research units from the Foundation's scientific network involved: LGDP

Sub-thematic axis: IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*),

Objectives:

The aim of this project is to understand the molecular bases of virulence of phytopathogenic fungi. Plant pathogens like bacteria, oomycetes and fungi secrete numerous proteins during infection which serve to manipulate host defense and to exploit plant resources. These so-called effector proteins are therefore central elements in microbial virulence. In bacteria, a certain class called type III effectors are directly injected into host cells. In fungi, this system does not exist. While, some secreted proteins seem to accumulate and act inside host cells. With the objective of understanding to which extent and by which mechanisms they act as effectors of virulence and contribute to fungal pathogenicity, investigations will analyze proteins that the rice pathogenic fungus *Magnaporthe grisea* secretes into plant tissue during infection.

Functional analysis of fungal effector proteins is severely hampered by their huge number and their apparent functional redundancy. Fungal strains mutated for individual effectors are generally not altered in their virulence and it is necessary to develop functional screening systems to elucidate their role in fungal virulence and to decipher their mode of action.

In the present project, a high throughput screening system for the functional analysis of fungal candidate effector proteins based on the use of the bacterial rice pathogens *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv *oryzicola* (*Xoc*) will be developed. It will allow the identification of *Magnaporthe* effector proteins that have a strong impact on virulence or act as avirulence proteins.

Multiple indications now suggest that virulence effectors from one organism can function in other species even if they are phylogenetically unrelated. Fungal candidate effector proteins will thus be analysed for their role in virulence by expressing them as a fusion with a signal for bacterial type III secretion system in *Xoo* and *Xoc*. *Magnaporthe* candidate effectors are then added to the arsenal of *Xanthomonas* virulence factors. Pathogenicity assays will enable the analysis whether this affects (negatively or positively) the capacity of *Xanthomonas* to colonize host tissue and to cause disease symptoms.

Fungal effector proteins showing interesting phenotypes in those functional assays will be analyzed in more detail, e. g. by localizing them during infection, by measuring their contribution to fungal virulence by reverse genetics or by identifying the plant processes they target and the plant target molecules they interact with.

Action carried-out and results obtained:

The aim of the project was the development of a heterologous system for the functional analysis of fungal candidate effector proteins. The initial project was to develop a system based on the use of the bacterial rice pathogens *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Xanthomonas oryzae* pv *oryzicola*

(Xoc) for the identification of *Magnaporthe oryzae* effector proteins with a strong contribution to virulence or acting as avirulence proteins. For the development of the system, translational fusions between the N-terminal parts of the *Xanthomonas* avirulence (Avr) proteins AvrBs2 or AvrXa10 comprising signals for secretion by the bacterial type III secretion system (T3SS), and intracellularly acting *M. oryzae* Avr proteins Avr-Pita, Avr-Pia, AvrPii, Avr-Pik, Avr-Pizt and AvrCO39 were expressed in Xoo and Xoc. It was expected that these strains became avirulent on rice varieties carrying the cognate resistance genes due to specific and intracellular recognition of Avr proteins injected by Xoo or Xoc. However, even if fusion proteins were produced properly by Xoo, as demonstrated by western blot experiments, they did not confer avirulence indicating that they were not functional. Most propably this may be due to endogenous Xoo and Xoc effectors acting as potent suppressors of rice defence and suppressing resistance induced by *M. oryzae* Avr proteins. Due to these negative results, development of the Xoo- and Xoc-based system will not be pursued.

An in vitro system allowing to determine the capability of pathogen effectors to cross plant plasma membranes has been developed by the group of Prof. Tyler. Recombinant fusion proteins between effectors suspected to be translocated into host cells and GFP are produced in E. coli and added to roots of in vitro grown plants. Translocated effectors accumulate inside root cells resulting in fluorescent staining of the plant cytoplasm. A collaboration with the group of Prof Tyler was established in order to identify potentially translocated *M. oryzae* effectors. Three out of six tested *M. oryzae* effectors accumulated inside root cells, suggesting their translocation into host cells during rice infection. Host cell translocation of one of these effectors named PWL2, has been recently demonstrated by life cell imaging supporting significance of the results of the in vitro translocation assay.

Prospects for the future:

At present, additional *M. oryzae* effectors are tested for translocation in the in vitro system and motifs necessary and sufficient for translocation of *M. oryzae* effectors are searched. In addition, complementary experiments aiming to confirm translocation of candidate effectors by independent approaches, in particular life cell imaging, are under way.

Total Agropolis Fondation funding: € 36,452 (equipment, travel expenses, financial support for 2 master students)

Funding categorie(s): Agropolis Fondation small grants (support for small exploratory, risky and innovative projects)

Project duration: 15 October 2008 – 31 December 2010

Keywords: rice – fungus – virulence – secretion – *Xanthomonas* – effector