Development of a high-throughput system for the functional analysis of virulence effector proteins of Magnaporthe grisea

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ABSTRACT

: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.

Keywords : Plant, Operation/adaptation, Protein/proteomic, Rice

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PERSPECTIVES

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.