PROPOSITION DE STAGE M2 RECHERCHE 2019-2020

TITRE : Identification of novel pathogenicity genes by transposon-based insertional mutagenesis in *Zymoseptoria tritici*, a fungal pathogen of wheat

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Nombre de thèses en cours : 0
Possibilité de poursuite en doctorat : ▌OUI NON
Présentation de la proposition de stage : OUI NON
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INTRODUCTION, CONTEXTE SCIENTIFIQUE :

Studies on the molecular mechanisms involved in fungal pathogenicity on plants were successful in identifying infection-related regulatory networks and effectors (Lo Presti et al. 2015; Kou et al. 2016; Motaung et al. 2017; Collemare et al. 2019). Identification of novel mechanisms without "a priori" can be performed using transposon-based insertional mutagenesis (Dufresne et al. 2010). This type of mutagenesis uses an autonomous transposon mobilized in a heterologous species, allowing its insertion at random locations in host genome. Most genes identified by insertional mutagenesis are involved in functions not predictable with existing knowledge, opening new research fields.

In fungi, transposon tagging is mostly performed with *impala*, a *Tc1-mariner* transposable element from the fungal plant pathogen *Fusarium oxysporum* (Dufresne et al. 2010). This type of mutagenesis relies on excision vectors containing an autonomous copy of *impala* inserted in the 5'UTR of *A. nidulans* nitrate reductase gene (*niaD*). These vectors are introduced in a nitrate reductase deficient mutant of studied fungal species. *impala* excision events are selected by recovering nitrate-utilizing revertants resulting from the excision of *impala* from *niaD* 5'UTR. Most excision events (90%) are associated with the re-insertion of *impala* into host genome. *impala* transposon-tagging was successful in identifying genes involved in pathogenicity on plants, development, and growth in a large set of fungi (Dufresne et al. 2010). We have recently showed that *impala* can be mobilized in the wheat fungal pathogen *Z. tritici* (INRA Bioger in collaboration with Syngenta). *impala* excision from *niaD* and re-insertion in *Z. tritici* genome occurred at a high rate (80%). Analysis of *impala* insertion sites showed a positive bias for insertions in promoters and 5'UTRs of *Z. tritici* genes (72%), near transcription start sites. This peculiar insertion pattern is promising for activation tagging. This type of mutagenesis uses a chimeric transposon carrying a strong or a controllable promoter. Insertion of this chimeric transposon near a gene can lead to its over-expression. We have constructed an excision vector for activation tagging in which a strong constitutive promoter (*pGpd*) was inserted at the 3' end of *impala.* This chimeric *impala:Gpd* transposon was able to excise and re-insert in *Z. tritici* genome. Characterization of a few insertion sites showed that *impala:Gpd* inserted mostly in 5'UTRs and promoters of *Z. tritici* genes, as native *impala*. Half of these insertions may lead to gene over-expression.

PROJET DE RECHERCHE :

The objective of this research project is to identify novel genes involved in pathogenicity on plants using activation tagging. The project will use *impala* vectors previously developed for activation tagging in *Z. tritici*. Transformants carrying these vectors are available, and they will be used to increase the number of activation-tagging revertants. Revertants will be studied at the molecular level (re-insertion site) and screened for mutants (morphology, pathogenicity). The expression of genes located near *impala* re-insertion site will be monitored to detect over-expression mutants. A collection of 50 activation-tagging revertants has been already obtained, and a first screen identified 3 pathogenicity mutants, all corresponding to genes not known yet to be involved in infection. These existing and forthcoming pathogenicity mutants will be validated genetically (reverse genetics, complementation) to demonstrate that *impala* insertion is responsible of the observed mutant phenotype. Confirmed pathogenicity genes will be studied to decipher their role in infection (cellular biology, expression and functional studies).

1- Building a collection of activation tagging mutants in *Z. tritici*

The strong constitutive fungal promoter p*Gpd* (Dufresne et al. 2010) was used to construct *impala*-based activation tagging vectors. An activation tagging *impala* vector (*niaD::impala::Gpd*) was introduced in *Z. tritici* NIA15 nitrate reductase deficient mutant by *Agrobacterium* mediated transformation (ATMT). We have obtained 20 transformants producing revertants corresponding to *impala* excision and re-insertion events. These transformants will be used to obtain additional revertants (300) that will be characterized for their excision and re-insertion patterns.

2- Characterization of *impala::Gpd* revertants for their insertion sites and over-expression patterns
impala re-insertion sites will be characterized by LM-PCR using existing protocols in either existing revertants (50) or novel revertants produced in Part 1. Revertants resulting from the insertion of *impala* in a promoter or a 5'-UTR will be characterized for the possible over-expression of flanking genes. We expect to obtain a collection of at least 100 over-expression revertants.

3- Phenotyping of *impala impala::Gpd* revertants
Revertants from Part 2 will be tested for their morphology (color, development), and their growth on different media. These revertants will be also tested for their pathogenicity on wheat leaves using existing infection protocols. Revertants differing from wild type will be studied in Part 4.

4- Genetic analysis of *impala::Gpd* candidate pathogenicity mutants
Existing (3) and forthcoming (Part 3) candidate pathogenicity mutants will be validated genetically. The gene tagged by *impala* will be inactivated in wild type by targeted gene replacement. A wild type allele of this gene will be used to complement *impala* mutant. In case of over-expression mutants, the gene tagged by *impala* will be over-expressed in wild type. The phenotypes of these transformants will be compared to those of *impala* mutant and wild type. If the gene tagged by *impala* (inactivation, over-expression) is responsible of the mutant phenotype, it is expected that some of these targeted transformants display the same phenotype as *impala* mutant.

5- Functional analysis of pathogenicity genes identified by activation tagging
Genes confirmed as involved in pathogenicity (part 4) will studied for their role in infection. The infection process of the corresponding activation tagging mutants will be monitored using GFP tagged strains. The expression pattern and cellular localization of these genes during infection will be also examined using qRT-PCR and GFP/RFP translational fusions. Depending on the putative cellular function of each gene, functional studies will be conducted to study the role of these proteins in infection.

APPROCHES METHODOLOGIQUES :
General microbiological methods for fungal storage, growth, purification, transformation, and morphological/growth assays are available. Protocols for pathogenicity assays on wheat leaves are available. Methods for cloning into ATMT vectors (BIOGER cloning platform) and *Z. tritici* ATMT transformation are available. Vectors for activation-tagging (*impala::Gpd)* are available. Transgenic *Z. tritici* strains carrying activation-tagging vectors are available. A first collection of revertants was obtained and will be extended in the framework of the project. Molecular analysis of *impala* excision and re-insertion events will performed using existing PCR protocols. *impala* re-insertion sites will be characterized using existing LM-PCR protocols. Revertants resulting from the insertion of *impala* in a promoter or a 5'-UTR will be further characterized for the expression level of genes flanking *impala* insertion site using qRT-PCR. Vectors for targeted gene replacement, complementation and over-expression will be constructed using available ATMT vectors (BIOGER cloning platform).

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