INTERNSHIP PROPOSAL M2 RESEARCH 2025-2026

TITLE: Dissecting a New Gene-for-Gene Interaction Between Wheat and _Zymoseptoria tritici_

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INTRODUCTION, SCIENTIFIC CONTEXT:

The phytopathogenic fungus _Zymoseptoria tritici_ causes Septoria tritici blotch (STB), one of the most damaging foliar diseases of wheat worldwide. Fungicides targeting STB represent a major share of fungicide use and costs in European cereal production. Deploying wheat varieties with genetic resistance is therefore a highly desirable, environmentally friendly, and complementary approach to disease control. For this reason, STB has become a major breeding target, and the average resistance level to STB in French bread wheat varieties has steadily increased since 2005 (Gravé _et al._, 2024). However, _Z. tritici_ possesses a strong adaptive capacity, due to its mixed reproduction system (sexual and asexual), short generation time, and very large population sizes. These features often lead to a rapid breakdown of resistance, thereby limiting its long-term effectiveness.

Resistance to STB is highly polygenic and involves numerous gene-for-gene (GFG) interactions between resistance genes in the host and avirulence genes in the pathogen, which may have either qualitative or quantitative effects on the interaction (Langlands-Perry _et al._, 2023). In most cases, the resistance genes present in French bread wheat varieties remain unknown, and the corresponding fungal avirulence genes are even less well characterized. Our research aims to identify the plant and fungal genes involved in GFG interactions in this pathosystem, with particular emphasis on those relevant to French elite wheat varieties. Knowledge of these genes would support detection and pyramiding of resistance genes in new varieties, as well as monitoring of resistance breakdown and evolution of virulence in fungal populations.

To genetically map resistance genes in the host or avirulence genes in the pathogen, different approaches are used that link phenotypic variation with underlying genetic variation in selected plant or fungal populations. One approach is quantitative trait locus (QTL) mapping, performed in progeny populations derived from crosses between parents with contrasting phenotypes. For example, Langlands-Perry _et al._ (2022, 2023) used reciprocal QTL mapping in biparental wheat and fungal populations to uncover GFG interactions. They identified the resistance gene _Stb20q_ on wheat chromosome 5D and functionally validated its corresponding avirulence gene _AvrStb20q_ in _Z. tritici_. Such functional validation is essential to establish the role of candidate pathogenicity genes in host-pathogen interactions. Another powerful approach is genome-wide association study (GWAS), which exploits genetic diversity

in a population of unrelated individuals (Sanchez-Vallet *et al.*, 2018; Amezrou *et al.*, 2024).

RESEARCH PROPOSAL:

We applied GWAS to a collection of 126 French isolates sampled in 2018 from four bread wheat cultivars with contrasting resistance levels: Apache, Rubisko, Fructidor, and LG-Absalon. Pathogenicity was evaluated on the same cultivars, leading to the identification of 11 candidate pathogenicity genes. One of them, encoding a small secreted protein typical of fungal effectors, was specifically associated with cultivar Rubisko. This gene was deleted in an avirulent isolate via *Agrobacterium*-mediated transformation, and phenotyping of the mutants is underway to confirm its role in pathogenicity. Our working hypothesis is that this effector functions as an avirulence gene interacting with an unknown resistance gene in Rubisko.

You will extend this project by confirming whether the candidate pathogenicity gene functions as an avirulence gene and by identifying the corresponding resistance gene in cultivar Rubisko.

METHODOLOGIES:

Your work will include:

1/ Construction of complementation mutants in the candidate gene knockout background, using ectopic integration of both the avirulent and virulent alleles of the candidate gene via *Agrobacterium*-mediated transformation;

2/ Quantitative phenotyping under controlled conditions of the available knockout mutants, as well as the complementation mutants carrying either the avirulent or virulent allele, on a susceptible wheat cultivar and on Rubisko;

3/ QTL mapping in the Rubisko × Rebelde progeny population already available at BIOGER: incl. preparing seeds for genotyping the population with the TaBW35K array, phenotyping for resistance against an isolate carrying the avirulent allele, and mapping resistance QTL using R/qtl.

RESEARCH UNIT:

The INRAE BIOGER Research unit (BIOlogy of fungal plant pathogens: from GEnomes to agRo-ecosystems) is a French center of excellence for research into fungal diseases affecting field crops (wheat, rapeseed) and vines. BIOGER develops multidisciplinary approaches (ranging from epidemiology to molecular biology and genomics) and multi-scale approaches (from genes to landscapes). Our main models are pathogens responsible for fungal diseases of major economic importance. Our work is organized into four main research areas: (i) Biological and ecological traits of phytopathogenic fungi, (ii) Fungal infection processes, (iii) Adaptation to the host and control methods, and (iv) Sustainability of fungal disease control strategies. The overall objective of our research is to meet societal expectations in terms of sustainable and environmentally friendly control of fungal diseases in field crops and management of the risks associated with the spread and adaptive potential of phytopathogenic fungi.

REFERENCES (maximum 5):

Amezrou R, Ducasse A, *et al.* (2024). Quantitative pathogenicity and host adaptation in a fungal plant pathogen revealed by whole-genome sequencing. *Nature Communications* 15(1): 1933.

Gravé J, Du Cheyron P, Audigeos D, Cabeza-orcel P, Baudart C, Chaillet I, Meleard B, Cohan J-P (2024). Les variétés de céréales à paille face aux maladies. *Perspectives Agricoles* 518. https://www.perspectives-agricoles.com/recherche-agronomie/les-varietes-de-cereales-paille-face-aux-maladies

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Langlands-Perry C, Pitarch A, *et al.* (2023). Quantitative and qualitative plant-pathogen interactions call upon similar pathogenicity genes with a spectrum of effects. *Frontiers in Plant Science* 14.

Sánchez-Vallet A, Hartmann FE, *et al.* (2018). Nature's genetic screens: using genome-wide association studies for effector discovery. *Molecular Plant Pathology* 19(1): 3-6.