Exploring gene diversity in wheat-*Zymoseptoria tritici* interactions as a path to improved Septoria tritici blotch control

Context

Septoria tritici blotch (STB), caused by the fungal pathogen *Zymoseptoria tritici*, is one of the most devastating diseases in France and Europe. This pathogen induces leaf necrosis, reducing photosynthesis and yield by an average of 1.5 tons per hectare per year. In the current agro-ecological context, sustainable management of STB primarily involves implementing integrated pest management (IPM) strategies, with the use of resistant wheat cultivars as the main pillar.

To date, 23 *Stb* genes and over 300 QTL conferring resistance to *Z. tritici* have been mapped on the wheat genome. Three *Stb* genes - *Stb6*, *Stb15*, and *Stb16q* - have been cloned. These genes encode receptor-like protein kinases anchored at the plasma membrane, which detect the presence of the pathogen extracellularly. In the early 2000s, it was shown that resistance mediated by the *Stb6* gene follows a gene-for-gene interaction with the corresponding avirulence gene from *Z. tritici*, *AvrStb6*. More recently, similar interactions have been observed for other *Stb/AvrStb* genes, raising questions about the universality of this interaction model. On the pathogen side, four *AvrStb* genes have been cloned: *Avr3D1*, *AvrStb6*, *AvrStb9*, and *AvrStb20q*. The exact mechanism (direct or indirect) by which AvrStb proteins are recognized by Stb proteins is currently unknown. A wide number of haplotypes has already been described for these *AvrStb* genes that mediate varying efficiencies of resistance against the same *Stb* alleles. On the other side, genetic diversity on *Stb* genes has been revealed with for instance 22 different alleles of *Stb6* described in the literature but only one known to confer resistance. While the impact of *AvrStb* diversity on the outcome of the interaction has been investigated, the effect of diversity of *Stb* alleles has not been as thoroughly studied, limiting the available alleles for breeding resistant cultivars and understanding the Stb/AvrStb recognition.

The population of *Z. tritici* is genetically very diverse, with several million haplotypes per hectare of wheat. This pathogen undergoes several cycles of sexual reproduction per year, allowing it to evolve rapidly and potentially overcome resistant wheat cultivars. To mitigate this, one proposed strategy is to diversify resistances and deploy them with a faster turnover based on the frequency of corresponding avirulence genes in the natural fungal population. This strategy requires a thorough understanding of the diversity of Stb/AvrStb interactions, the mechanisms of recognition between wheat and *Z. tritici*, and comprehensive data on the number, distribution, and frequency of *AvrStb* genes in the natural population of *Z. tritici*. To this end, the PhD project will be divided in two main objectives:

1-Identification of new alleles of wheat *Stb* genes involved in resistance

The objective of this task is to thoroughly evaluate the genetic diversity of the *Stb* genes, identify alleles involved in wheat resistance to STB, and unravel the specificity of their interaction with the corresponding *AvrStb* genes. The PhD student will investigate the allelic diversity of the wheat genes *Stb6* and *Stb9*, two of our current model genes. A large collection of several hundred wheat accessions, primarily landraces, will be selected in collaboration with our local biological resource center from the ten thousand accessions that have been recently genotyped. These landraces will be chosen from the 11 clades used to retrace the evolutionary history of wheat. The extracellular domain of these two genes involved in pathogen recognition will be sequenced using new sequencing technologies available at the Gentyane platform. Phylogenetic relationships between the different haplotypes and their evolution through wheat migration routes will be analyzed.

Once the allelic diversity is identified, the PhD student will evaluate the resistance efficiency of each *Stb* allele (both those documented in the literature and those identified in this project) against

different alleles of the corresponding *AvrStb* genes. At least 10 *Z. tritici* isolates, known to carry different *AvrStb* alleles that are widely represented in the *Z. tritici* French population, will be used to phenotype wheat accessions carrying the different allelic versions of *Stb*. *Stb* alleles identified as potentially conferring resistance according to the phenotyping assays will be functionally validated through virus-induced gene silencing (VIGS) and innovative transient approaches in wheat and/or tobacco. Identification of new resistant haplotypes will help unravel extracellular domains involved in pathogen recognition and understand the molecular basis of Stb/AvrStb specificities. These alleles will also provide new genetic resources for breeding programs.

2- What is the diversity of *Z. tritici* genes mediating pathogen recognition by resistance genes present in the current French wheat cultivars

The objective of this task is to identify, on a large scale, the genes from *Z. tritici* involved in recognition by resistance factors present in current French wheat cultivars. The genetic diversity of *Z. tritici* present in the field is filtered when collected on wheat leaves according to the resistance genes present in the wheat cultivar. For instance, isolates collected from a wheat cultivar carrying the *Stb6* gene are expected to predominantly carry the virulent allele of *AvrStb6*. This filter can be used to identify fungal genome genes involved in recognition by wheat resistance by analyzing the loss of genetic diversity among isolates collected from the same cultivar compared to those collected from different cultivars.

In 2023, naturally infected leaves from over a hundred of the most recent French wheat cultivars were collected. Isolates will be extracted from these leaves to create a collection of virulent isolates against a range of resistant wheat cultivars. These isolates will be sequenced to identify genes likely involved in recognition by the resistance factors present in the wheat cultivars from which they were collected. Then, the genetic diversity of these genes will be evaluated in worldwide collections of *Z. tritici* isolates available at INRAE. The resistance genes present in the corresponding wheat cultivars will be identified in a parallel project in collaboration with breeding companies. The PhD student will then evaluate the specificity of recognition between wheat resistant genes and candidate genes identified from the fungal genome through phenotyping experiments under controlled conditions. These findings will provide an overview of the fungal genes and their diversity involved in the recognition by French wheat cultivars, enabling us to track their frequency in the natural *Z. tritici* population. These results will be presented to breeding companies to discuss how these large-scale discoveries of avirulence genes can be utilized to develop effective strategies to combat STB.

Supervisors and environment: The PhD student will be co-supervised by Cyrille Saintenac (INRAE GDEC, Clermont-Ferrand, cyrille.saintenac@inrae.fr) and Thierry Marcel (INRAE BIOGER, Palaiseau, thierry.marcel@inrae.fr). The location of the PhD student will be shared between Clermont-Ferrand and Palaiseau. INRAE GDEC benefits from the most advanced experimental facilities (technical and experimental platforms) with skills, tools, and world-class modern equipment, which allow for growing crops under controlled conditions (four growth chambers fully equipped to maintain conditions required for optimal *Z. tritici* infection), broadband genotyping and sequencing approaches and laboratories fully equipped to perform molecular experiments. INRAE BIOGER is a leading cross-disciplinary research unit dedicated to 'field-to-gene' studies of major fungal pathogens impacting key crops in French agriculture, with the aim of developing sustainable strategies to control these devastating diseases. The unit benefits from state-of-the-art experimental facilities, including new greenhouses, growth chambers for cultivating fungi and conducting pathology assays, microbiology and molecular biology labs, as well as platforms for cytology and bioinformatics. BIOGER is located on the Agro Paris-Saclay campus within the University of Paris-Saclay.

Profile of the candidate:
We are seeking for a highly motivated candidate with a background in phytopathology and genetic. A Master degree in one of these related fields is required.

How to apply:
Please send to cyrille.saintenac@inrae.fr and thierry.marcel@inrae.fr a CV including contact information from your previous experiences and a cover letter. The PhD is expected to begin between October 2024 and January 2025. Applications will remain open until a suitable candidate is selected.

Some key references from PhD supervisors related to the PhD project:

Amezrou R, Audéon C, Compain J, Gélisse S, Ducasse A, **Saintenac C**, Lapalu N, Louet C, Orford S, Croll D, Amselem J, Fillinger S, **Marcel TC.** 2023. A secreted protease-like protein in *Zymoseptoria tritici* is responsible for avirulence on *Stb9* resistance gene in wheat. *PLoS Pathog.* 19(5):e1011376. doi: 10.1371/journal.ppat.1011376

Amezrou R, Ducasse A, Compain J, Lapalu N, Pitarch A, Dupont L, Confais J, Goyeau H, Kema GHJ, Croll D, Amselem J, Sanchez-Vallet A, **Marcel TC** (2024) Quantitative pathogenicity and host adaptation in a fungal plant pathogen revealed by whole-genome sequencing. *Nat Commun.* 15(1):1933. doi: 10.1038/s41467-024-46191-1

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Saintenac C, Lee WS, Cambon F, Rudd JJ, King RC, Marande W, Powers SJ, Bergès H, Phillips AL, Uauy C, Hammond-Kosack KE, Langin T, Kanyuka K. 2018. Wheat receptor-kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen *Zymoseptoria tritici*. *Nat Genet.* 50(3):368-374.

Stephens C, Ölmez F, Blyth H, McDonald M, Bansal A, Turgay EB, Hahn F, **Saintenac C**, Nekrasov V, Solomon P, Milgate A, Fraaije B, Rudd J, Kanyuka K. 2021. Remarkable recent changes in the genetic diversity of the avirulence gene *AvrStb6* in global populations of the wheat pathogen *Zymoseptoria tritici*. *Mol Plant Pathol.* 22(9):1121-1133