

**INTERNSHIP PROPOSAL M2 RESEARCH 2026-2027****Molecular and phenotypic validation of fungal genes involved in the adaptation of *Zymoseptoria tritici* to bread wheat and durum wheat**

Supervisors: Ulysse GUILLOTEAU, Thierry MARCEL & Marie DUFRESNE (research team ADEP)

Address, phone, e-mail: INRAE BIOGER, Campus Agro Paris-Saclay, 22 Place de l'Agronomie - CS80022, 91120 Palaiseau – France, Tél. : +33 1 89 10 13 35, ulysse.guilloteau@inrae.fr, thierry.marcel@inrae.fr, marie.dufresne@inrae.fr

Introduction, scientific context.

Plant-pathogenic fungi represent a major threat to global food security. However, most fungal pathogens are able to infect only a limited range of plant species, a phenomenon known as “host species specificity”. Some fungal species may have a broad host range, while specialized subpopulations within these species are able to infect only one or a few host species. Even within a given fungal species, individuals may differ in their ability to infect different cultivars of the same plant species, a phenomenon referred to as “host cultivar specificity”. Characterizing the host range and degree of host specificity of major fungal pathogens of crops is important for better assessing and managing epidemic risks, including the movement of individuals or genes between fungal populations associated with different cropping systems. Understanding the genetic and genomic determinants underlying host specialization is also essential for assessing the risk of host shifts (*i.e.* the expansion of a pathogen's host range) and the potential breakdown of resistance deployed in crop varieties.

Zymoseptoria tritici is a fungal pathogen responsible for Septoria tritici blotch, an important foliar disease of both bread wheat (*Triticum aestivum*, hereafter BW) and durum wheat (*T. turgidum* var. *durum*, hereafter DW). This ascomycete has a mixed reproductive system combining sexual and asexual reproduction, generating high levels of genetic diversity and enabling its populations to rapidly adapt to selective pressures, including resistant wheat varieties and fungicides. Although *Z. tritici* is able to infect both bread and durum wheat, its populations are generally composed of individuals that show a high degree of specialization towards one or the other host species. We have observed this specialization in France and, more broadly, in several Mediterranean countries, both in production areas dominated by either BW or DW and in regions where the two crops are grown in close proximity. Our objective is to gain a better understanding of host specialization in *Z. tritici*, with a particular focus on the genetic and genomic determinants underlying the ability of this pathogen to infect bread versus durum wheat.

Research proposal.

To identify genes involved in the adaptation of *Z. tritici* populations to BW or DW, we assembled a large panel of several hundred isolates collected across the Mediterranean region from samples originating from both BW and DW. All isolates were whole-genome sequenced, allowing us to identify genetic polymorphisms, including SNPs and indels, among isolates. We then performed a genome-wide association study (GWAS) to identify associations between genetic polymorphisms and the host of origin of the isolates (BW or DW). This GWAS identified several genomic regions containing four candidate genes that may contribute to BW/DW specialization. Three genes encode small secreted proteins (SSP) with characteristics typical of fungal effectors, and one gene encodes a secreted peptidase that could also be involved in pathogenicity. One SSP has already been disrupted using an *Agrobacterium tumefaciens*-mediated transformation (ATMT) approach in two *Z. tritici* isolates originating from DW. The resulting mutant strains were then phenotyped on a panel of several BW and DW varieties. These experiments showed that disruption of this gene tended to reduce pathogenicity specifically on DW, independently of the wheat variety tested. However, this effect was quantitative and difficult to reliably detect under the controlled conditions used for our pathogenicity assays.



The objective of this internship is to pursue the investigation of the contribution of our candidate genes to the adaptation of *Z. tritici* to DW. The internship will combine molecular biology approaches to generate and characterize the mutant strains with plant pathology experiments to finely assess their phenotypes on panels of BW and DW varieties. This will provide an opportunity to investigate the genetic basis of host specialization in *Z. tritici* through a combination of functional genetics and phenotyping approaches.

Methodologies.

Your work will include:

- 1/ The wild-type and mutant strains for the first candidate gene, which are already available, will be used to phenotype a panel of durum wheat genotypes under controlled conditions. This will provide further insights into the role of this gene in pathogenicity on durum wheat, and enable an association genetic analysis (QTL or GWAS) to investigate whether resistance loci effective against the wild-type strain remain effective against the mutant strain.
- 2/ Disruption mutants will be generated for one or two of the remaining candidate genes, as well as double mutants in which two candidate genes are simultaneously disrupted. *Agrobacterium tumefaciens*-mediated transformation (ATMT) or CRISPR-Cas9 technology will be used to generate these mutants. Briefly, the target candidate genes will be replaced by a DNA cassette containing 5'- and 3'-fragments homologous regions interrupted by an antibiotic resistance marker for selection.
- 3/ The wild-type, single- and double-mutant strains will be quantitatively phenotyped under controlled conditions on a reduced panel of DW and BW genotypes. This will allow to determine whether the candidate genes contribute to pathogenicity on durum wheat and whether they interact synergistically to promote infection of DW.

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.

<https://bioger.versailles-saclay.hub.inrae.fr/>

References (maximum 5).

- Sanchez-Vallet A, Hartmann FE, Marcel TC, Croll D (2018). Nature's genetic screens: using genome-wide association studies for effector discovery (Opinion Piece). *Molecular Plant Pathology* **19**(1): 3–6.
- Langlands-Perry C, Pitarch A, Lapalu N, Cuenin M, Bergez C, Noly A, Amezrou R, Gélisse S, Barrachina C, Parrinello H, Suffert F, Valade R, Marcel TC (2023). Quantitative and qualitative plant-pathogen interactions call upon similar pathogenicity genes with a spectrum of effects. *Frontiers in Plant Science* **14**: 1128546.
- 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. *Nature Communications* **15**(1): 1933.