Genetics of pathogenicity in *Zymoseptoria tritici*

Thierry MARCEL – University Paris-Saclay, INRAE UR BIOGER, France

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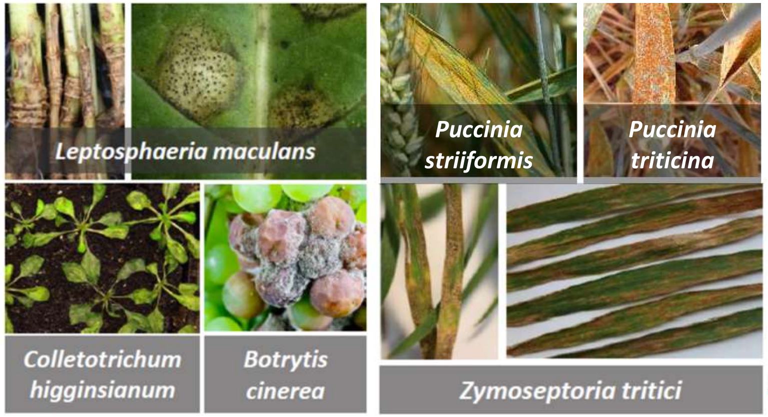
National Research Institute for Agriculture, Food and Environment

18 Research Centres
14 Scientific Departments
>200 Research Units
>12,000 Employees

Our overarching goal is to address societal expectations for **sustainable and environmentally friendly strategies in the fight against fungal diseases** in major crop plants, and to **manage risks associated with the dissemination and adaptive potential of phytopathogenic fungi**.

BIOGER Campus Agro Paris-Saclay

Reference research unit on fungal diseases of major European crops:



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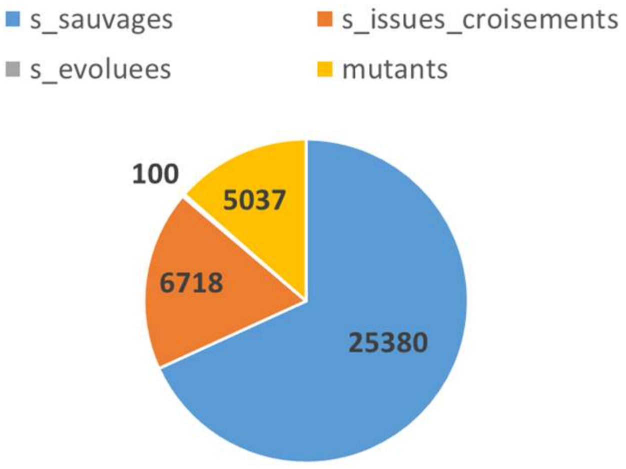
Inventory of 11 collections of fungal isolates:

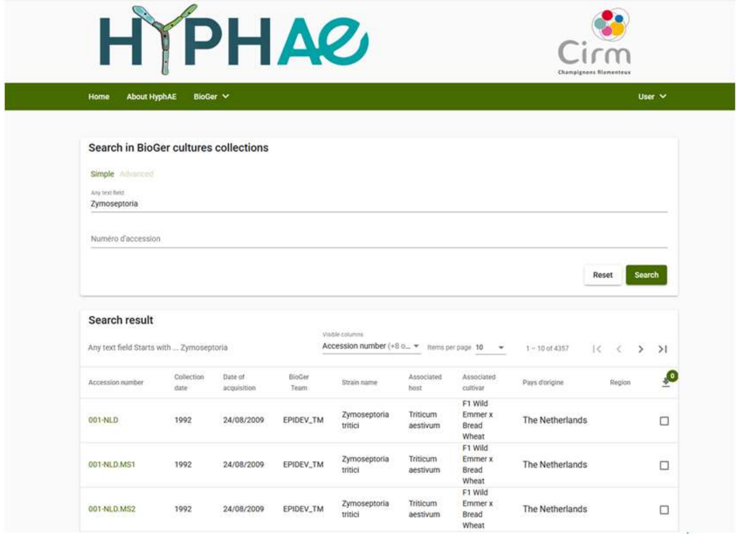
Collections	Référents	Genres concernés
AMAR	Clémentine Duplaix / Anne Sophie Walker	Botrytis Fusarium Microdochium Sclerotinia Ustilago Zymoseptoria Pyrenophora
BiogeR-syst	Valérie Laval / Noémie Vignolles	Fusarium Microdochium Parastagonospora
Botrytis	Muriel Viaud / Pascal Le Pêcheur	Botrytis
ECCP_Colleto	Richard O'Connell	Colletotrichum
EGIP	Anne Genissel / Marc-Henri Lebrun	Zymoseptoria
Epidémio_FS	Frédéric Suffert / Sandrine Gélisse	Zymoseptoria
Epidémio_HG	Henriette Goyeau	Puccinia
Epidémio_LG	Lilian Gout	Zymoseptoria Parastagonospora
Epidemio_ML	Marc Leconte	Puccinia
Epidemio_TM	Thierry Marcel	Zymoseptoria
EPLM	Mylène Balesdent / Laurent Coudard / Michel Meyer / Isabelle Fudal / Bénédicte Ollivier / Jessica Soyer / thésards	Leptosphaeria

Survey 2021 by Noémie Vignolles

37.235 strains



Collaboration with CIRM Filamentous Fungi Marseille



Search in BioGer cultures collections

Any text field: Zymoseptoria

Numéro d'accession

Search result

Any text field Starts with ... Zymoseptoria

Visible columns: Accession number (+8 o...

Items per page: 10

1 – 10 of 4357

Accession number	Collection date	Date of acquisition	BioGer Team	Strain name	Associated host	Associated cultivar	Pays d'origine	Region	0
001-NLD	1992	24/08/2009	EPIDEV_TM	Zymoseptoria tritici	Triticum aestivum	F1 Wild Emmer x Bread Wheat	The Netherlands		☐
001-NLD.MS1	1992	24/08/2009	EPIDEV_TM	Zymoseptoria tritici	Triticum aestivum	F1 Wild Emmer x Bread Wheat	The Netherlands		☐
001-NLD.MS2	1992	24/08/2009	EPIDEV_TM	Zymoseptoria tritici	Triticum aestivum	F1 Wild Emmer x Bread Wheat	The Netherlands		☐

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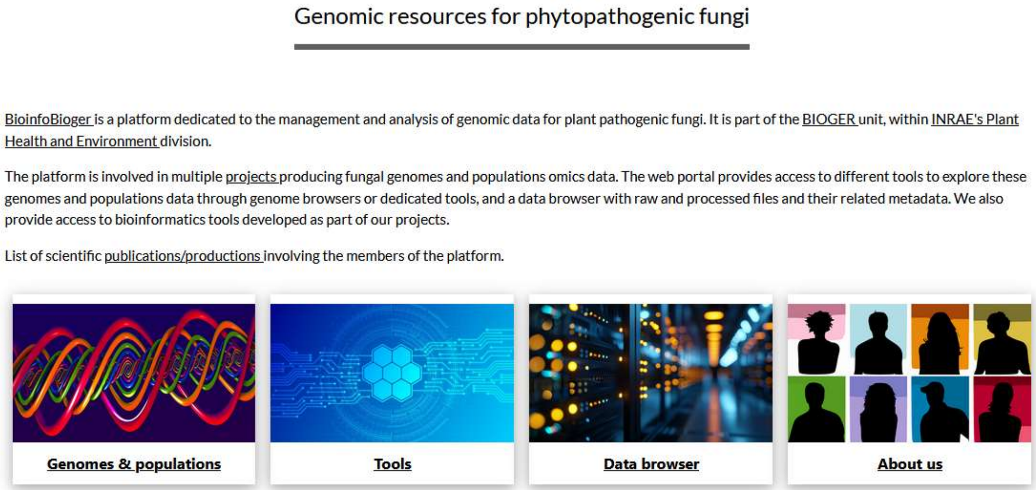
BIOINFOBIOGER: BIOGER Bioinformatics platform

https://bioinfo.bioger.inrae.fr/

LAPALU Nicolas

SIMON Adeline



Genomic resources for phytopathogenic fungi

BioinfoBioger is a platform dedicated to the management and analysis of genomic data for plant pathogenic fungi. It is part of the BIOGER unit, within INRAE's Plant Health and Environment division.

The platform is involved in multiple projects producing fungal genomes and populations omics data. The web portal provides access to different tools to explore these genomes and populations data through genome browsers or dedicated tools, and a data browser with raw and processed files and their related metadata. We also provide access to bioinformatics tools developed as part of our projects.

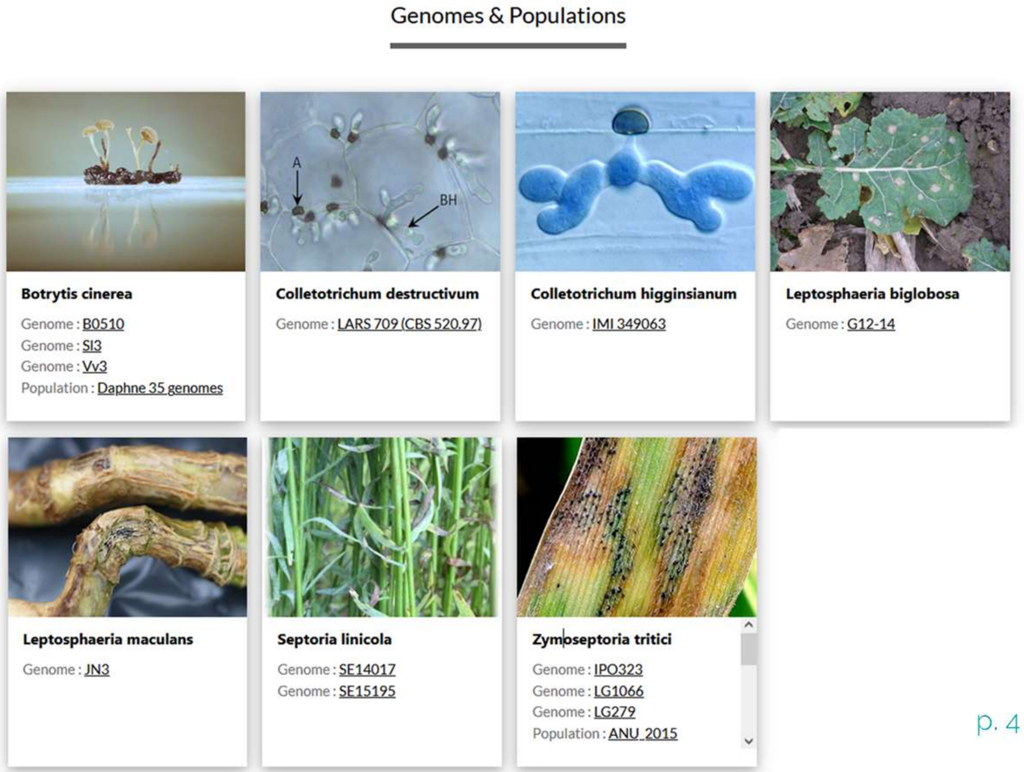
List of scientific publications/productions involving the members of the platform.

Genomes & populations

Tools

Data browser

About us



Genomes & Populations

Botrytis cinerea
Genome : B0510
Genome : SI3
Genome : Vv3
Population : Daphne 35 genomes

Colletotrichum destructivum
Genome : LARS 709 (CBS 520.97)

Colletotrichum higginsianum
Genome : IMI 349063

Leptosphaeria biglobosa
Genome : G12-14

Leptosphaeria maculans
Genome : JN3

Septoria linicola
Genome : SE14017
Genome : SE15195

Zymoseptoria tritici
Genome : IPO323
Genome : LG1066
Genome : LG279
Population : ANU_2015

INRAE BIOGER

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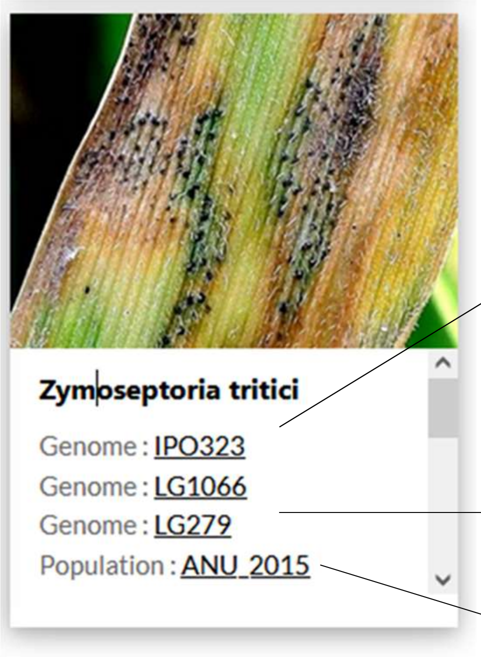


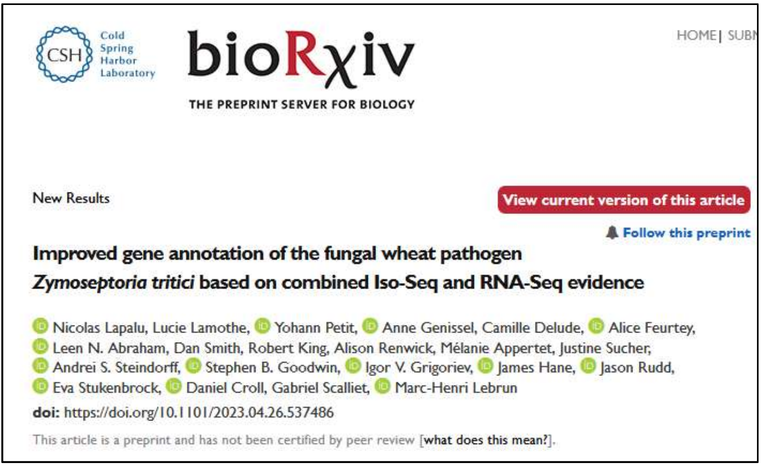
Zymoseptoria tritici

Genome : IPO323

Genome : LG1066

Genome : LG279

Population : ANU_2015



New Results

Improved gene annotation of the fungal wheat pathogen *Zymoseptoria tritici* based on combined Iso-Seq and RNA-Seq evidence

Nicolas Lapalu, Lucie Lamothe, Yohann Petit, Anne Genissel, Camille Delude, Alice Feurtey, Leen N. Abraham, Dan Smith, Robert King, Alison Renwick, Mélanie Appertet, Justine Sucher, Andrei S. Steindorff, Stephen B. Goodwin, Igor V. Grigoriev, James Hane, Jason Rudd, Eva Stukenbrock, Daniel Croll, Gabriel Scalliet, Marc-Henri Lebrun

doi: https://doi.org/10.1101/2023.04.26.537486

This article is a preprint and has not been certified by peer review [**what does this mean?**].

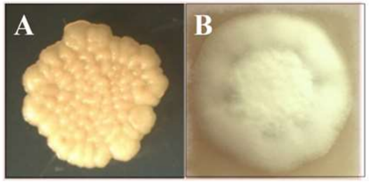
Two reference genomes of *Z. tritici* isolates specialized on durum wheat: INRA13-LG0279 & INRA13-LG1066

Whole genome Illumina sequences for 768 *Z. tritici* isolates (mostly French and European isolates), 321 of which have been pathotyped

Biology of *Zymoseptoria tritici*

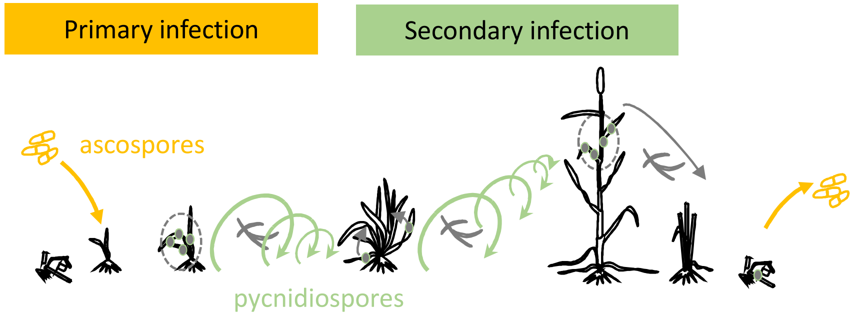
Cause the Septoria tritici blotch disease on wheat:

- Ascomycete (Dothideales)
- Dimorphic (yeast, mycelium)
- Heterothallic (Mat1, Mat2)
- Latent necrotroph



Dimorphism of *Z.tritici:*
A: yeast form
B: mycelium form

(Picture by Solweig LUCE)



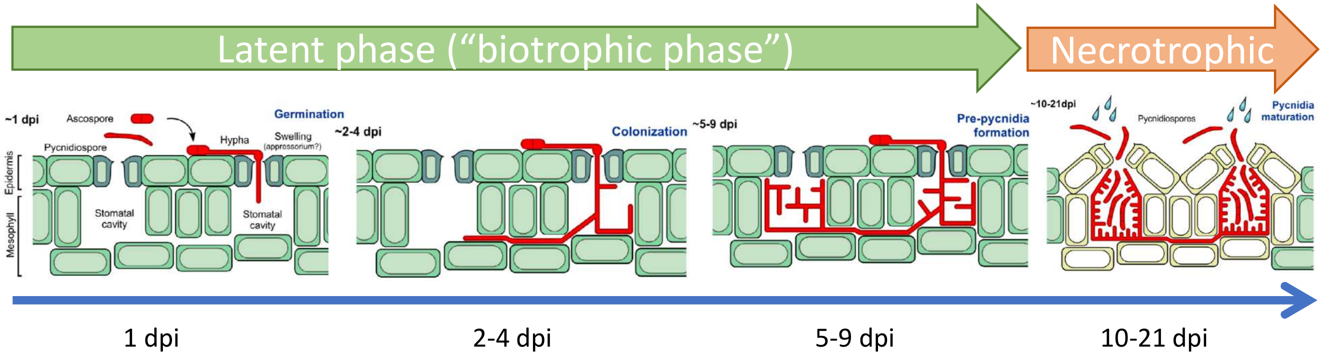
Primary infection

Secondary infection

ascospores

pycnidiospores

Development of a Septoria tritici blotch epidemic (Suffert *et al.*, 2015)



Latent phase (“biotrophic phase”)

Necrotrophic

1 dpi

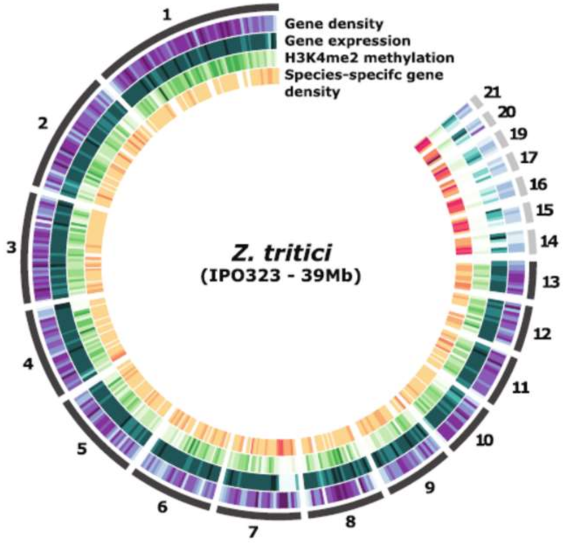
2-4 dpi

5-9 dpi

10-21 dpi

Plant infection stages of *Zymoseptoria tritici* (Steinberg, 2015)

Genetics of *Zymoseptoria tritici*



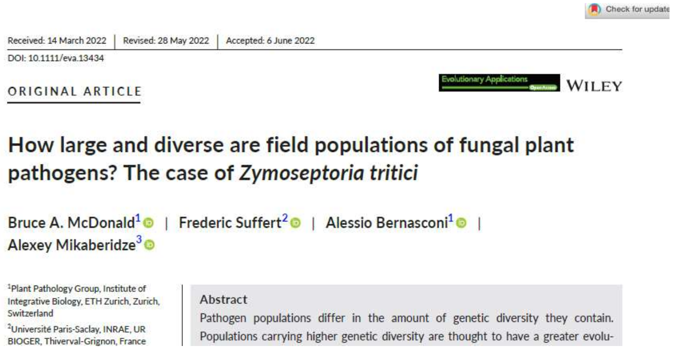
Feurtey *et al.* 2020

- Haploid (n)
- Genome size of 39.7 Mbp
- 13 core and 8 accessory chromosomes
- 13,414 re-annotated gene models

High level of genetic diversity

"We found that wheat fields experiencing typical levels of infection are likely to carry between 3.1 and 14.0 million pathogen genotypes per hectare and produce at least 2.1–9.9 trillion pycnidiospores per hectare."

Controlled crosses between isolates



Check for update

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DOI: 10.1111/eva.13434

ORIGINAL ARTICLE

Evolutionary Applications WILEY

How large and diverse are field populations of fungal plant pathogens? The case of *Zymoseptoria tritici*

Bruce A. McDonald[1] | Frederic Suffert[2] | Alessio Bernasconi[1] |
Alexey Mikaberidze[3]

[1]Plant Pathology Group, Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland

[2]Université Paris-Saclay, INRAE, UR BIOGER, Thiverval-Grignon, France

Abstract

Pathogen populations differ in the amount of genetic diversity they contain. Populations carrying higher genetic diversity are thought to have a greater evolu-

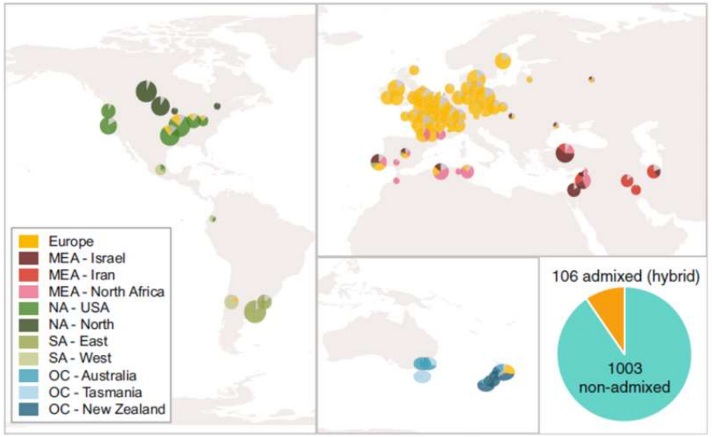
McDonald *et al.* 2022

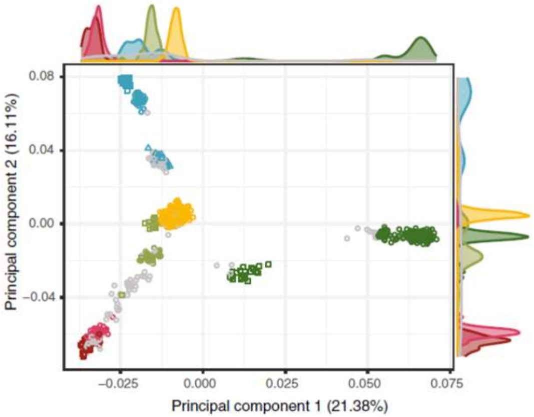
Orellana-Torrejon *et al.* 2022

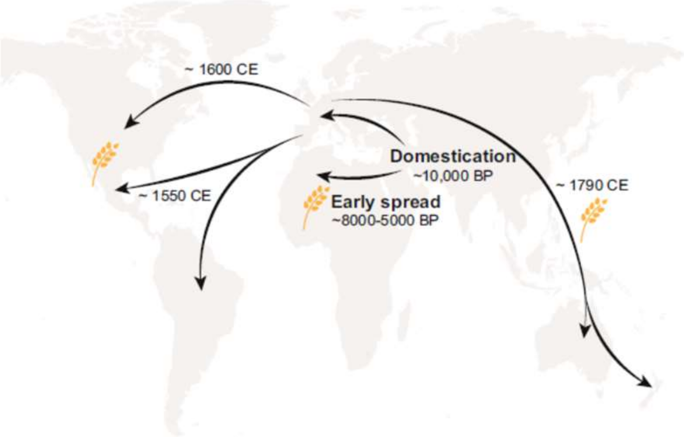
Population genomics of *Z. tritici*

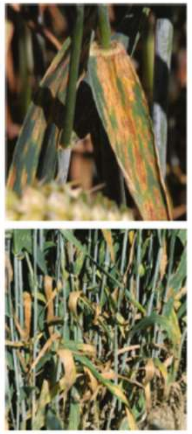
Worldwide genetic structure based on 1109 genomes:

Feurtey *et al.* (2023), Nature Communications 14









Eleven genetic clusters with differentiation mostly at the continental level:

- global genetic structure of the pathogen reveals multiple founder events associated with the introduction of wheat to new continents.

Global expansion of *Z. tritici* retracing the historical spread of its host:

- increased activity of transposable elements,
- significant standing variation for adaptation to new climates encountered during the global spread.

Genetics of pathogenicity in *Z. tritici*

Three reasons why it is crucial to identify pathogenicity factors in crop fungal pathogens:

1. Understanding Mechanisms of the Infectious Process

- How the pathogen infects, colonizes, and damages the host?
- Key molecular mechanisms such as effector functions, toxin production, and immune evasion.

2. Enhancing Durable Resistance in Crops [Effector Assisted Breeding]

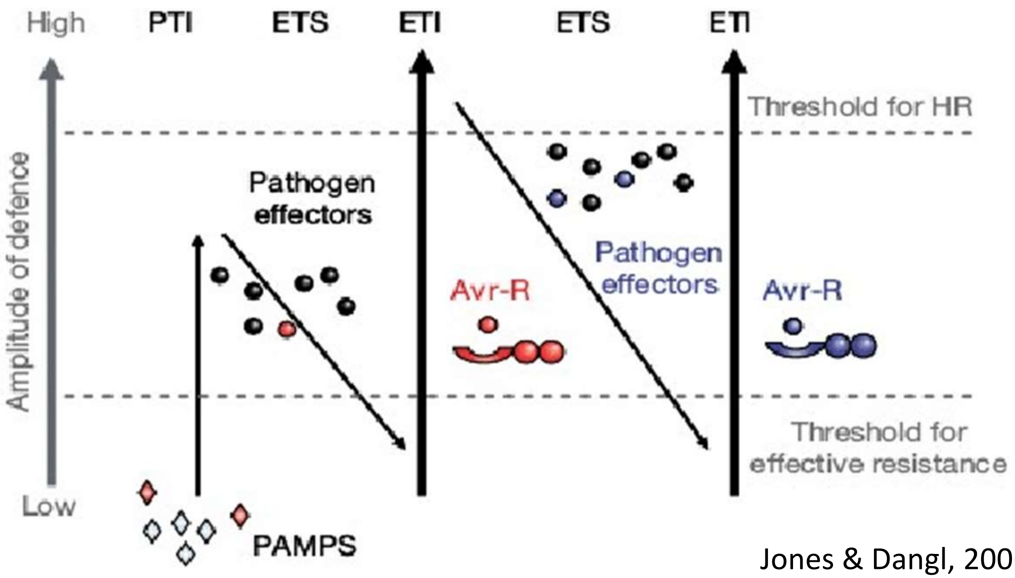
- Use knowledge on fungal effectors to identify new resistance genes, and to help cloning.
- Develop wheat varieties with stronger and more durable resistance.
- Engineer disease resistance genes.

3. Monitoring and Predicting Pathogen Evolution

- Track virulent strains and understand how fungal populations adapt to host resistances.
- Forecast future outbreaks and emerging threats.
- Improve the strategic deployment of resistance genes.

Genetics of pathogenicity in *Z. tritici*

The zig-zag model in plant-pathogen interactions:



Jones & Dangl, 2006

« Detection of an effector by an R protein triggers rapid activation of a very effective defense. »

Pathogen effectors: molecule, typically a protein or small RNA, secreted by a fungal pathogen to manipulate the host plant's cellular processes and facilitate infection.

Avirulence genes: gene in a pathogen that encodes a product, typically an effector protein, that can be recognized by a corresponding resistance (R) gene in the host plant, triggering a defense response.

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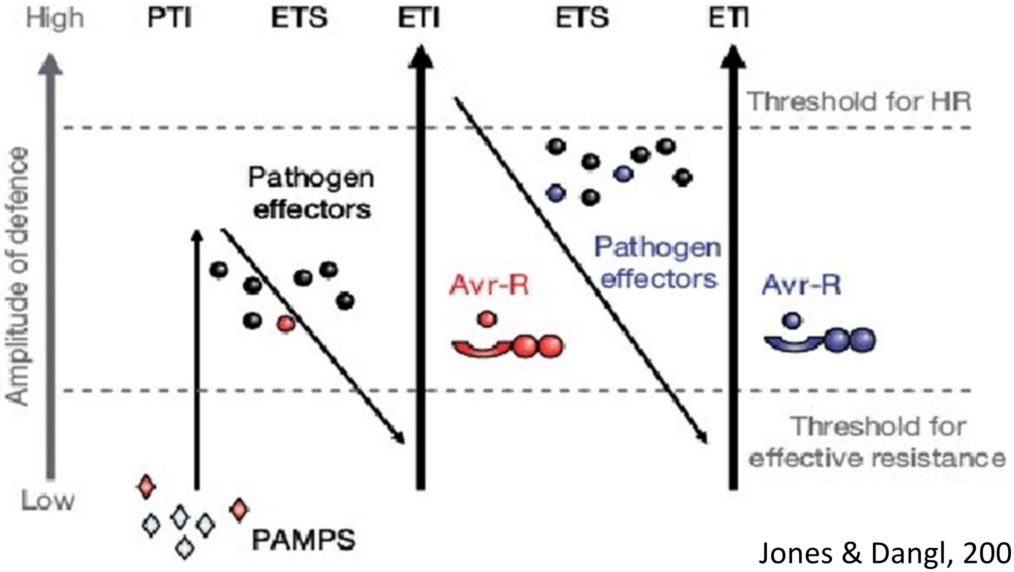
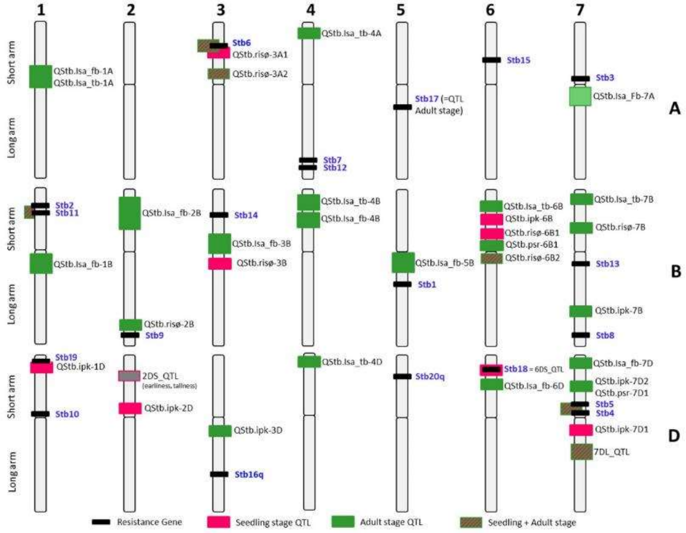


Jones & Dangl, 2006

« Detection of an effector by an R protein triggers rapid activation of a very effective defense. »

Genetic map of resistances to *Z. tritici* in bread wheat:



by Rothamsted Research

23 *Stb* resistance genes and 100s of QTLs

Are only *Stb* resistance genes involved in gene-for-gene interactions?

Genome Wide Association Studies in *Z. tritici*

Dissection of host-pathogen interactions using GWAS

GANDALF

ANR Bioadapt 2013-17

Panel of 103 *Zt* isolates
collected on Bread Wheat
cultivars Apache and Premio

Phenotyped for agressiveness
on 12 *Stb* carrying BW cultivars

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BASF 2019-22

Panel of 126 *Zt* isolates collected
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Phenotyped for aggressiveness on 4
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BASTAFUN

INRAE SPE 2023-25

Panel of 238 *Zt* isolates collected
on Bread and Durum Wheats
cultivars in EuroMediterranean

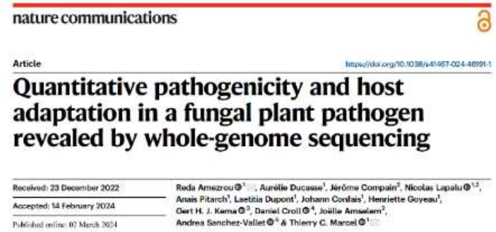
Phenotyped for temperature
adaptation and for aggressiveness

ENDURO

CTPS CASDAR 2020-23

Panel of 106 *Zt* isolates collected
on most cultivated **Durum Wheat**
cultivars in Europe

Phenotyped for aggressivenes on 16
DW and 4 BW cultivars



nature communications

Article https://doi.org/10.1038/s41467-024-46191-1

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Received: 23 December 2022
Accepted: 14 February 2024
Published online: 02 March 2024

Reda Amezrou[1], Aurélie Ducasse[1], Jérôme Compain[2], Nicolas Lapalu[1,2], Anais Pitarch[1], Laetitia Dupont[1], Johann Confais[1], Henriette Goyeau[1], Gert H. J. Kema[3], Daniel Croll[4], Joëlle Amselem[2], Andrea Sanchez-Vallet[5] & Thierry C. Marcel[1]

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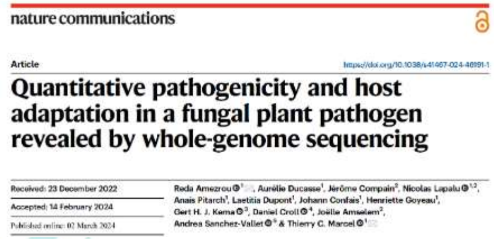

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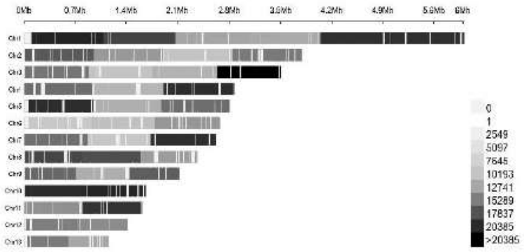
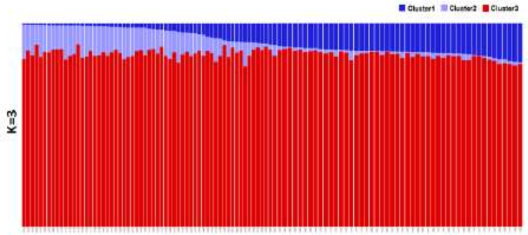
WGS of **109** diverse *Zt* isolates resulted in **718,810** genome-wide SNPs after quality filtering

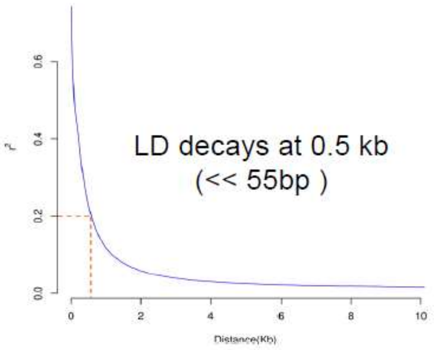


High-density polymorphism
(1SNP every 55bp)

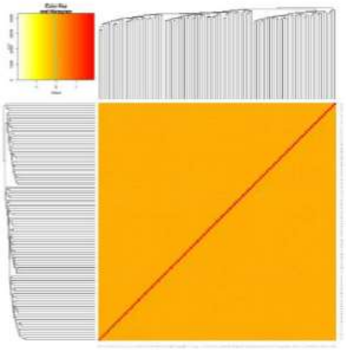


No population structure
(lowers the rate of false positives)

IDEAL FOR GWAS



LD decays at 0.5 kb
(<< 55bp)



No relatedeness among isolates

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Agence Nationale de la Recherche ANR

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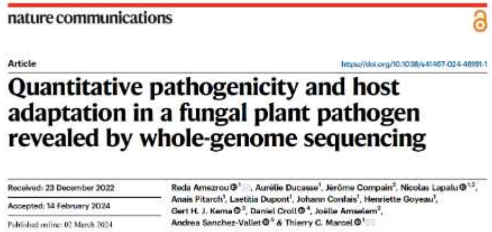

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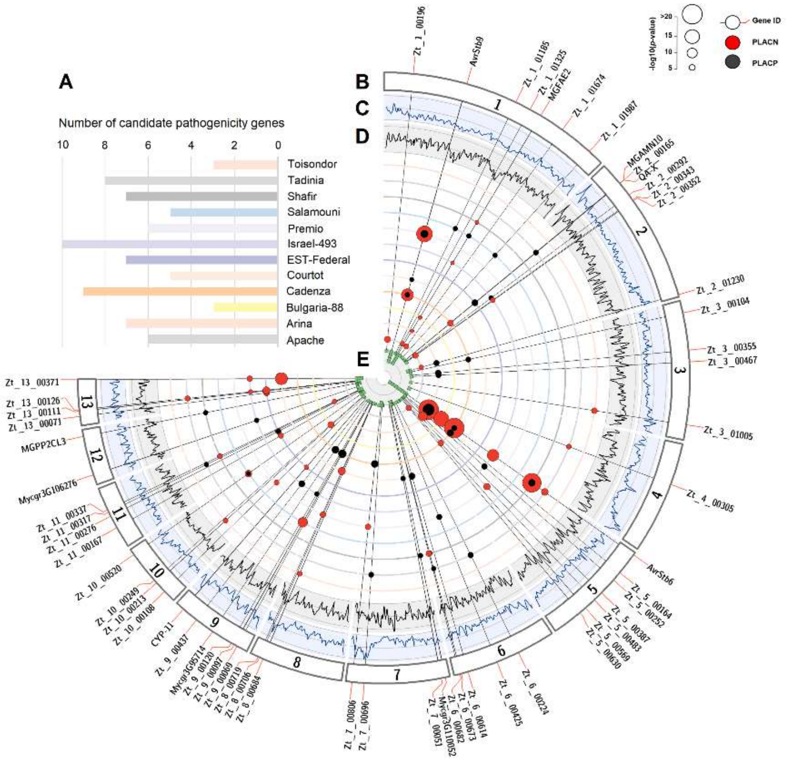
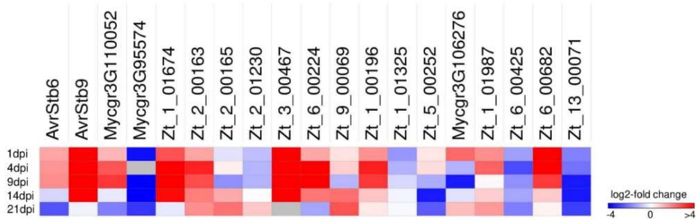


Amezrou *et al.* (2024), Nature Communications

Detection of 69 candidate genes, including nineteen genes highly expressed and/or differentially expressed *in planta*:



> Pathogenicity in *Z. tritici* has a complex polygenic architecture and is predominantly quantitative.

> Virulence-associated genes are mostly host specific.

Genome Wide Association Studies in *Z. tritici*

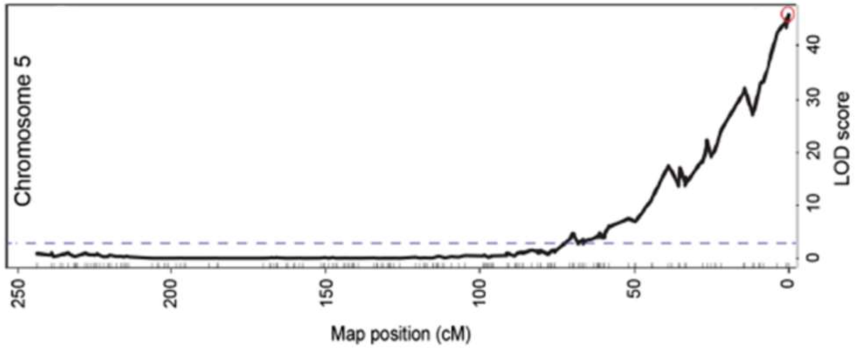
Cloning of ***AvrStb6***, responsible for avirulence on wheat cultivars carrying the resistance *Stb6*

AvrStb6 encodes a small secreted protein of 63 amino acids and rich in 11 cysteine residues

> The *Stb6-AvrStb6* interaction lead to a strong and asymptomatic disease resistance reaction

Zhong *et al.* (2017), New Phytologist

- Identified by QTL mapping



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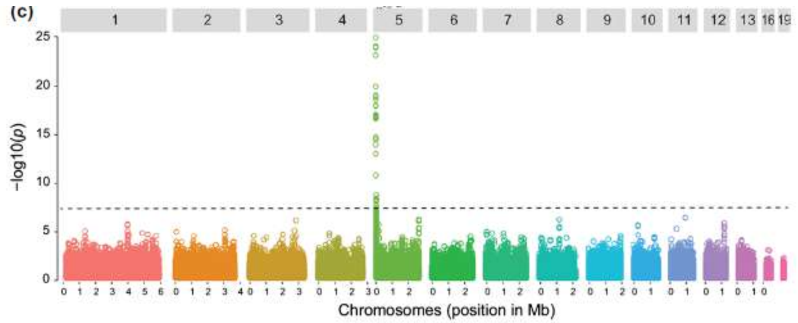
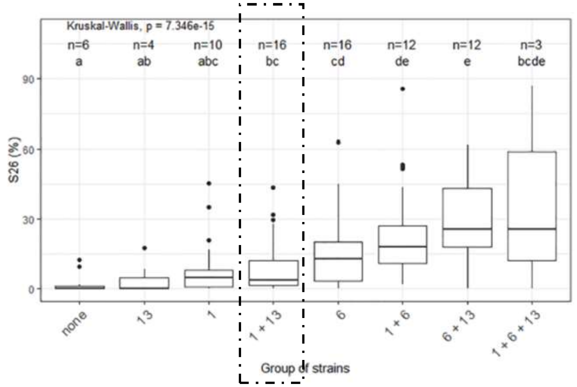


Genome Wide Association Studies in *Z. tritici*

Cloning of ***AvrStb20q***, responsible for avirulence on wheat cultivars carrying the resistance *Stb20q*

AvrStb20q encodes a small secreted protein of 78 amino acids and rich in 11 cysteine residues



AvrStb20q

> The *Stb20q-AvrStb20q* interaction lead to a quantitative reduction in disease symptoms

Langlands-Perry *et al.* (2023), Frontiers in Plant Science

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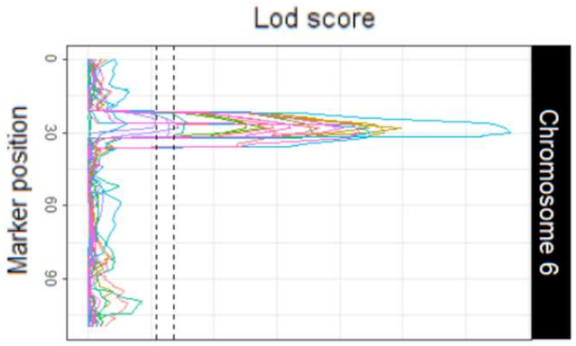

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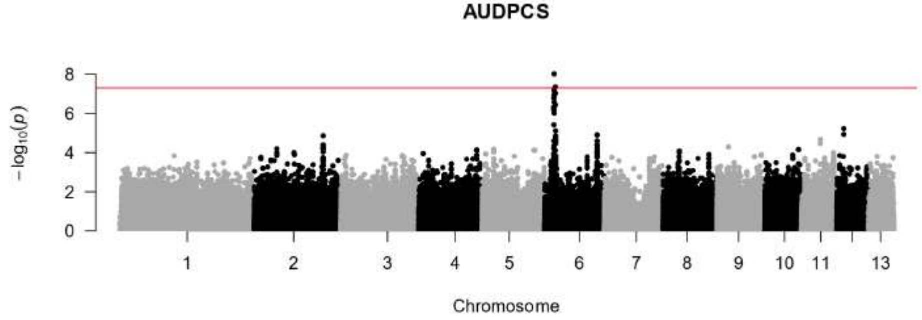

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TC Marcel, UR BIOGER
14/03/2025 – Groupe Céréales, Gembloux

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Phenotyped for aggressiveness on 4 BW cvs and for fungicide resistance

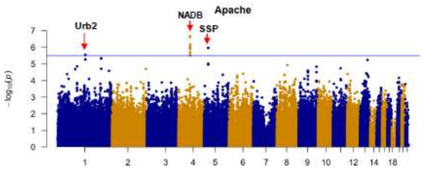
Aggressiveness on the 4 wheat cultivars in controlled conditions

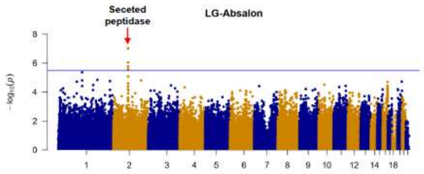
LG-Absalon
Fructidor
Rubisko
Apache

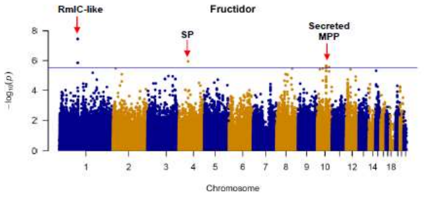
STB resistance

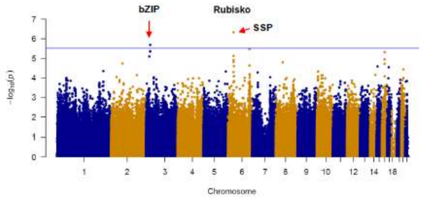
Percentage of leaf surface covered by:
- necrosis
- pycnidia

Measured at 14, 20, 26 days post inoculation





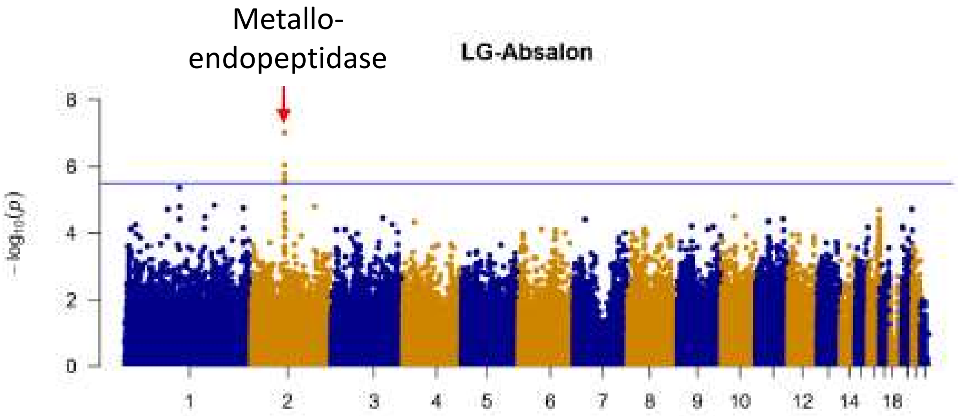


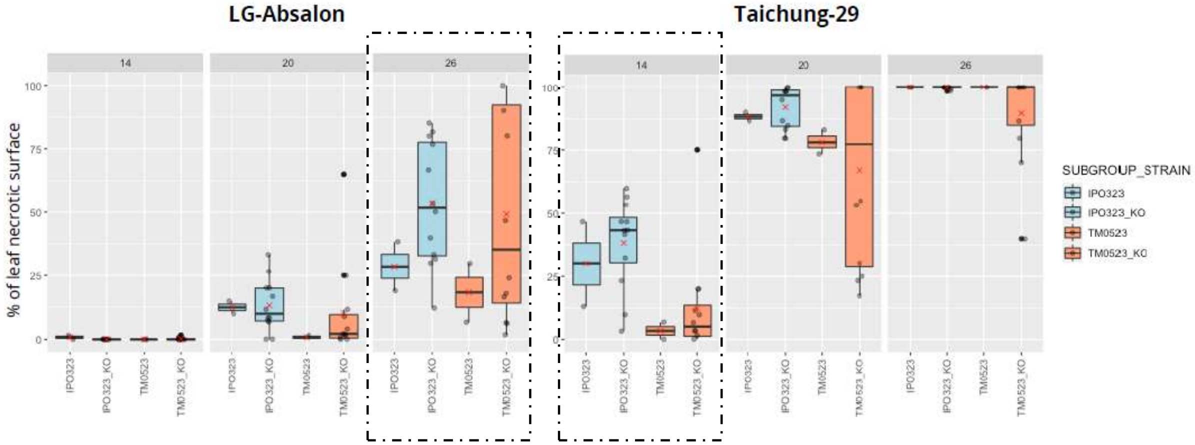


> Detection of 11 candidate genes

Genome Wide Association Studies in *Z. tritici*

Cloning of a **metallo-endopeptidase** involved in *Z. tritici* pathogenicity





The peptidase-encoding gene was identified by GWAS only on the cultivar LG Absalon

➡

> The deletion of the peptidase-encoding gene tends to increase aggressiveness of *Z. tritici* independently of the cultivar

Genome Wide Association Studies in *Z. tritici*

VISA
BASF 2019-22

Panel of 126 *Zt* isolates collected on Bread Wheat cultivars Apache, Rubisko, Fructidor, LG Absalon

Phenotyped for aggressiveness on 4 BW cvs and for fungicide resistance

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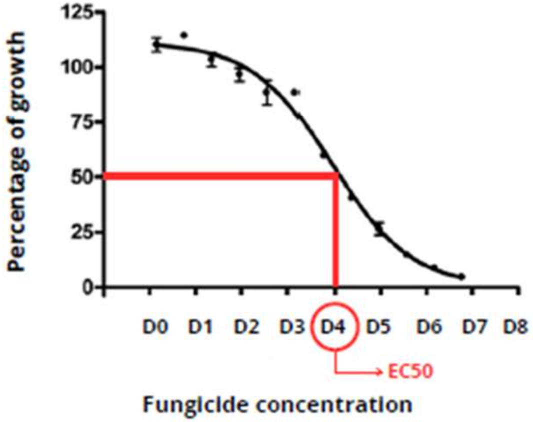
Fungicide resistance

4 DMIs (demethylation inhibitors)
2 SDHIs (succinate dehydrogenase inhibitors)



Adapted from Hanane Jary

Genome Wide Association Studies in *Z. tritici*

Construction of a correlation matrix between aggressiveness and fungicide resistance traits:

VISA

BASF 2019-22

Panel of 126 *Zt* isolates collected on Bread Wheat cultivars Apache, Rubisko, Fructidor, LG Absalon

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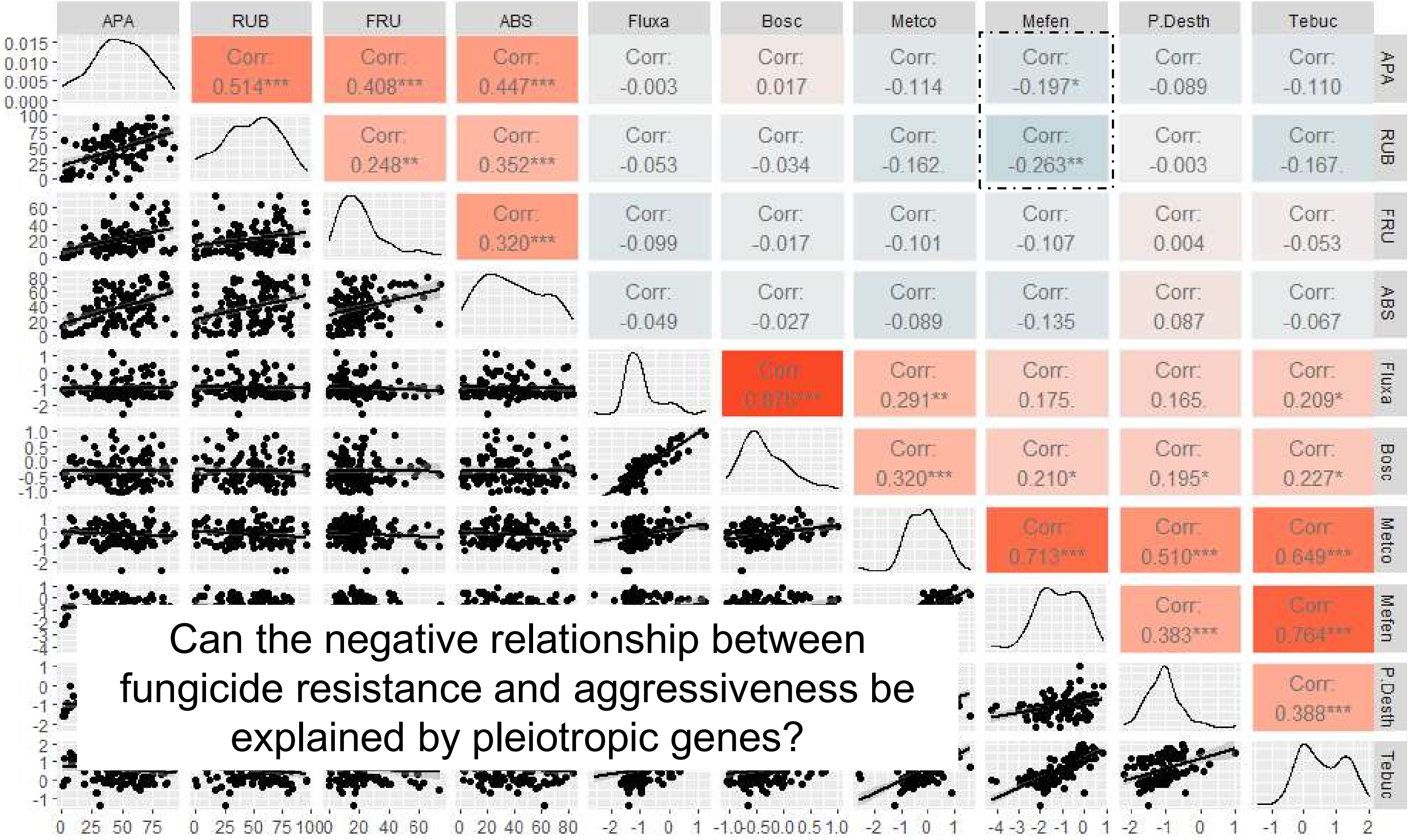
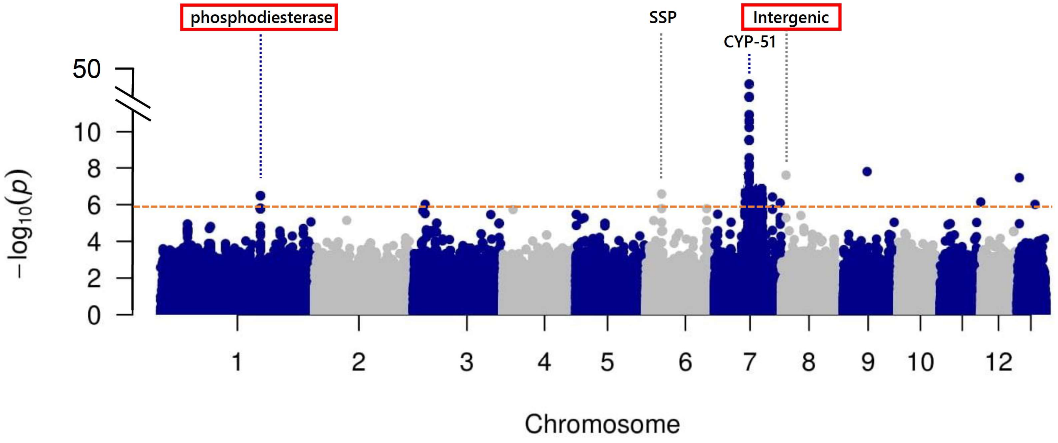
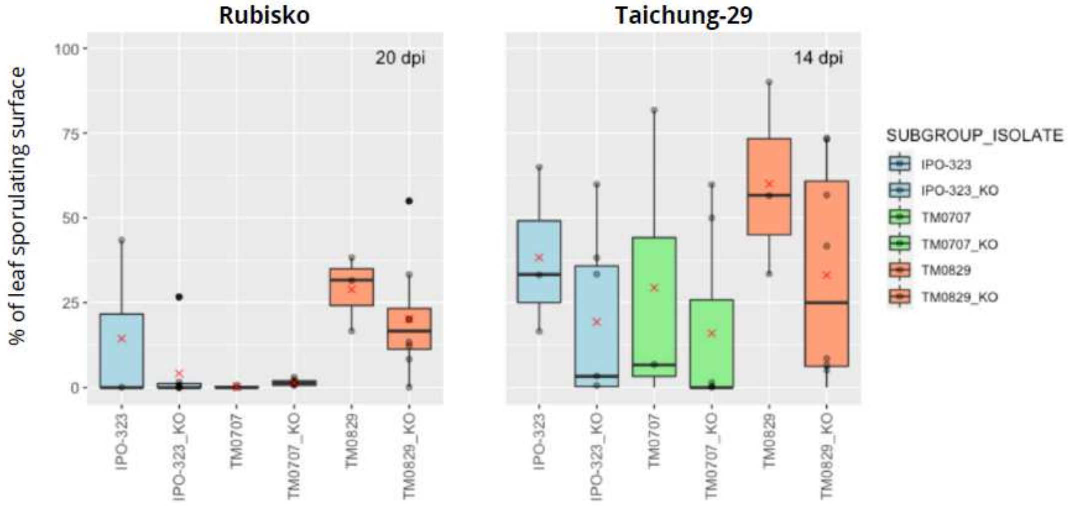


Can the negative relationship between fungicide resistance and aggressiveness be explained by pleiotropic genes?

Genome Wide Association Studies in *Z. tritici*

The metaCCA algorithm (Cichonska *et al.*,2016) allows a powerful univariate genotype multivariate phenotype assocation testing, which is useful for correlated traits.





Phosphodiesterases (PDE) are regulators of cyclic nucleotide signaling (cAMP and cGMP). In fungi, PDE was shown to be involved in metabolism, stress responses, azole sensitivity and virulence etc.

> The PDE gene acts as a quantitative pathogenicity factor and is potentially involved in fungicide sensitivity in *Z. tritici.*

Take-home messages

In the wheat – *Z. tritici* pathosystem,

- Pathogenicity has a complex polygenic architecture and is predominantly quantitative
- Gene-for-gene interactions can underlie qualitative (*AvrStb6*) and quantitative (*AvrStb20q*) interactions
- Fungal populations exhibit standing genetic variation in pathogenicity genes (metallo-endopeptidase) associated with aggressiveness, regardless of the host cultivar
- A phosphodiesterase gene may have antagonistic pleiotropic effects constraining adaptation of the fungus

Perspectives

- Validation of new avirulence and pathogenicity genes on-going…
- Construction of a pangenome graph for *Z. tritici* to improve the detection of candidate genes
- GWAS for pathogenicity of *Z. trititi* on durum wheat (PhD thesis)
- Allelic diversity of known *Stb* and *AvrStb* genes (PhD thesis)
- Development of high-throughput tools to follow the evolution of pathogenicity genes in fungal populations

Thank you…

Funders

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INRAE BIOGER

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Adaptive and epidemiological processes in wheat-fungal pathogen interactions

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