VIP

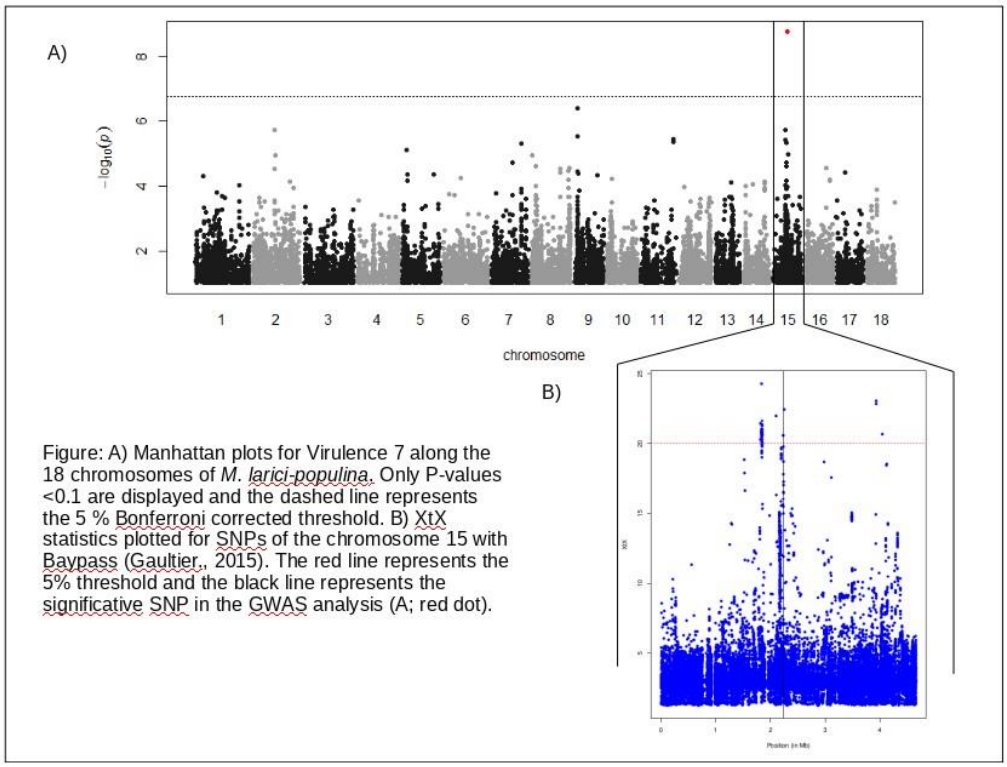

Figure: A) Manhattan plots for Virulence 7 along the 18 chromosomes of *M. larici-populina*. Only P-values <0.1 are displayed and the dashed line represents the 5 % Bonferroni corrected threshold. B) XtX statistics plotted for SNPs of the chromosome 15 with Baypass (Gaultier., 2015). The red line represents the 5% threshold and the black line represents the significative SNP in the GWAS analysis (A; red dot).

Virulence Investigations in the Poplar-Poplar rust pathosystem: from population genomics approach to protein characterization of the virulence factor responsible for the resistance R7 breakdown

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Work package: *WP1*

Context – *Melampsora larici-populina*, responsible for the poplar rust, is a major threat for the poplar culture. This pathogenic fungus has overcome all the qualitative resistance (noted R1 to R8) deployed so far. The major event occurred in 1994 with the breakdown of the resistance R7. This event led to the invasion of Western Europe by a virulent population 7 (i.e. capable of infecting poplar cultivars carrying the resistance R7) in replacement of the pre-existing pathogenic populations (Persoons et al., 2017).

Objectives – The main objective of the project is to identify the genetic determinism of virulence 7. The molecular evolution of host-pathogen interactions follows a gene for gene model where two specific loci interact with each other (Flor , 1971). A genetic change at one locus can cause loss of recognition on the plant side, and therefore lead to a gain in virulence on the pathogen side. The objective here is to identify within the genome of *M. larici-populina* the locus as well as the genetic changes responsible for the R7 breakdown.

Approaches – The evolutionary scenario linked to the R7 breakdown is an ideal situation to identify the molecular basis of this adaptation, in particular the intensity of the selection event and its contemporary history allow us to have access to genomics data on individuals collected before, during and after this breakdown. We used a genome scan approach to detect specific genomics regions of a certain population (here the virulent population). This method is based on a combination of neutrality tests designed to detect allelic frequency biases induced by the rapid and recent fixation of a mutant (selective sweep) in the virulent population 7 specifically. The results were then compared to a pan-genomic association analysis (GWAS) explicitly testing the link between virulence profiles and SNP polymorphism in order to identify the candidate locus for virulence 7.

Key results – - The genome scan based on the population structure identified several SNPs with significant values on chromosome 15 which could be linked to virulence 7.
- GWAS analysis confirms one of these SNPs on chromosome 15 and locates it in an exon of a gene.
- This candidate avirulence gene has certain characteristics of canonical effectors (coding for a protein rich in cysteine, specific to *M. larici-populina*, expressed early and of unknown function), and would have a secretory but unconventional signal.
- More remarkably: two independent but complementary mechanisms are responsible for virulence 7: a non-synonymous SNP and a deletion of the locus.

Main conclusions including key points of discussion –
The genome scan and the GWAS both pointed to the same locus, strengthening its candidate gene status and validating the joint use of the two methods (with or without a priori). As the genome analysis focuses on the population structure and the GWAS on the phenotype of each isolate analysed, we ensure that the candidate gene explains both the major evolutionary change in this species and is also responsible for the evolution of the phenotype which has been observed in *M. larici-populina* after the R7 breakdown.
To our knowledge, this is the first case in a diploid organism where resistance breakdown can be explained by two different mechanisms (a deletion and a non-synonymous SNP) on the same candidate effector. For a diploid organism, this double determinism makes it possible to appreciably increase the probability of being phenotypically virulent because each allele of virulence taken independently is recessive.

Perspectives –
Our study would identify the first avirulence gene in this obligatory biotrophic fungal species. The mutagenesis approach being particularly delicate in this species, we are going towards the production of corresponding protein and the test of interaction in a heterologous system.

Valorization –
- Presentation at the MPMI 2019 congress.
- Two publications have been written and will be submitted by the end of January.
- A chapter popularizing the work on poplar rust has just been published in the book “Immunité des Plantes” (Plant Immunity) published by QUAE (ISBN 2759232336).

Leveraging effect of the project –
Two PhD projects are on-going on molecular characterization (C. Louet) and eco-evolutionary modeling (M. Saubin). The ANR project Endurance has been resubmitted (call for proposal 2021).