RUSTOMICS

An integrated multi-omics approach to decipher the poplar-poplar rust interaction

Principle investigator: Sébastien Duplessis, UMR Interactions Arbres/Micro-organismes (IAM) *1136*

LabEx partners: Projet multi-équipes INRAE/UL au sein de l'UMR IAM (équipes *Réponse aux stress et régulation redox*, *Ecologie des champignons pathogènes forestiers* et *Ecogénomique des interactions*)

Thematic action concerned: *WP1*

Context —
Rust diseases caused by fungi of the order Pucciniales cause significant damage to many crops and plantation trees such as poplar. The pathosystem established between poplar and Melampsora larici-populina is one of the most advanced models for the understanding of the mechanisms of interaction with Pucciniales, in particular because of the availability of host and pathogen genomes. Studies conducted in the last ten years have allowed progress in our understanding of the infectious process, however, our knowledge of the molecular mechanisms at play still needs to be improved.

Objectives —
In order to clarify the determinants of infection in the fungus and the resistance and defense mechanisms implemented by poplar during the interaction with the pathogen, we propose to set up a multi-omics approach in the context of the infection of a poplar cultivar by avirulent (resistance process) and virulent (successful infection) isolates.

Approaches —
Based on time-course infection produced in the lab (collection of 7 points between spore inoculation and symptoms at 7 days), mRNAs and small-RNAs will be extracted in order to perform a transcriptomic analysis by Illumina sequencing, proteins will be extracted in order to perform a proteomic analysis, and finally, metabolites will be extracted in order to complete the analysis by a metabolomic approach.

Key results —
• Poplar-*M. larici-populina* interaction kinetics have been generated and have already enabled i) a proteomic analysis and ii) a metabolomic analysis carried out in dedicated platforms. The results are currently being processed (see preliminary proteomic results below).
• RNAs are currently being extracted for transcriptomic analysis as part of Jean Mourot's Master 2 internship (M2 AETPF-IPE, UMR IAM). Training courses dedicated to transcriptomic analysis in R have been conducted to exploit the results.
• Shotgun proteomic analysis of 63 samples from infection kinetics of poplar leaf discs infected with the fungus *M. larici-populina* [compatible/disease, incompatible/resistance and control conditions at 7 incubation times after inoculation - 0, 6, 12, 24, 48, 96, 168 h x 3 replicates) was carried out at the *Plateforme d'Analyse Protéomique de Paris Sud-Ouest* (PAPPSO, Gif-sur-Yvette, Mélisandre Blein-Nicolas & Marlène Daventure). This analysis revealed 40,067 peptides corresponding to 4,454 proteins. Semi-quantitative analysis by Spectral Count, an approach in which proteins are quantified on the basis of the number of MS2 spectra assigned to them after the identification stage, showed that of these 4,454 proteins, 406 were significant to the condition effect, 1,351 were significant to the time effect and 367 were significant to both the time and condition effects (*P*-value < 0.05). Quantitative analysis based on XIC (eXtracted Ion Chromatograms), an approach that summarizes the peptide intensities measured by the mass spectrometer into protein abundances, revealed 270 proteins significant for the condition effect, 1,401 for the time effect and 117 for both the time and condition effects (*P*-value < 0.05). More detailed analyses to aggregate all these data are currently underway.

Main conclusions including key points of discussion —
Data acquisition is progressing at a satisfactory pace, in line with the experimental plan initially set out in the project. We already note that fungal abundances remain limited (dilution of pathogen molecules in host tissues), as expected.

Perspectives & Valorization —
Transcriptomic data should be acquired around summer 2024, and will be processed rapidly during the summer using a dedicated R pipeline currently in preparation. The last half of 2024 will be devoted to establishing correlations between the three approaches and valorizing the results in the form of a publication.

Leveraging effect of the project —
The transcriptomic data generated in the *Rustomics* project will be integrated into an ANR project (submission 2024) for a broader transcriptomic analysis of the entire life cycle of the fungus. In addition, the transcripts obtained will contribute to improving the annotation of a version 3 of the *M. larici-populina* genome currently being obtained as part of the *Graine d'Artemis PRETE* project.