INTEGRARUST

Molecular Bases of the R-Avr Interaction in the Model Forest Pathosystem *Populus-Melampsora* through an Integrative Biology Approach

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Context — The strong impact of rust diseases on the productivity of many agrosystems is urging us to understand the mechanisms of interaction between rust fungi and their host plants in order to design durable resistance strategies. Our knowledge of rust fungus biology is still limited, mostly because of the obligate biotrophic status of these pathogens, which impedes the development of functional approaches. Thanks to recent advances made in genomics, it is now possible to dissect complex genetic traits such as disease resistance in non-model species. The availability of the genome sequence of both the poplar tree *Populus trichocarpa* and the poplar leaf rust fungus *Melampsora larici-populina*, is a great opportunity to decipher the genetic bases of tree-biotrophic fungus interactions.

Objectives — The present project aims to study the mechanisms of interaction between molecular determinants responsible for resistance (*R* genes) in poplar and virulence (effectors, *Avr* genes) in the poplar rust fungus.

Approach — We propose to develop an integrative biology approach bridging population genomics, functional genomics and protein biochemistry to identify the key determinants of fungal virulence and to validate the interaction between R and AVR proteins.

Key results —

- Genomes of 88 *M. larici-populina* isolates from a large collection sampled over 20 years in France around a major event of rust resistance breakdown were sequenced in collaboration with the Joint Genome Institute. Single Nucleotide Polymorphisms (SNPs) were identified in these genomes by comparison to the *M. larici-populina* reference genome. Structure of targeted populations and demographic events related to *M. larici-populina* virulence 7 emergence were determined by population genetics.

- Based on the population structure knowledge, a Genome Scan approach was applied to identify genomic regions under selection possibly after the virulence 7 emergence event.

- Our panel of approaches to perform the functional analysis of selected candidate rust effectors has been enriched through 3 key collaborations: transient expression of *M. larici-populina* candidate effectors fused to fluorescent markers in the plant heterologue system *Nicotiana benthamiana* in collaboration with The Sainsbury Laboratory in UK, recombinant protein expression in *Pichia pastoris* in collaboration with Bourgogne University and the structural analysis of effectors by NMR in collaboration with INSERM/CNRS in Montpellier.

Main conclusions including key points of discussion — The Genome Scan analysis has identified genomic regions under selection related to the virulence 7 emergence, including candidate genes that now need to be functionally addressed for validation.

Future perspectives — Candidate genes will be scrutinized in the coming 2 years to determine the genes more likely responsible for virulence 7 (e.g. expression during interaction with the host plant). Recombinant proteins corresponding to the most promising candidate genes will be produced and infiltrated in poplar cultivars carrying the Resistance 7 to validate the R-Avr interaction (hypersensitive response). If successful, the fungal protein function will be studied moreover (localisation and protein targets in host cells) and the corresponding poplar Resistance protein will be searched by co-immunoprecipitation and pull-down. The collaborations established in the frame of the IntegraRust project will allow progress in the understanding of the role of the candidate effectors revealed by the populational approach.

Valorisation —

Publications

Persoons A, Morin E, Delaruelle C, Payen T, Halkett F, Frey P, De Mita S, Duplessis S (2014) Patterns of genomic variation in the poplar rust fungus *Melampsora larici-populina* identify pathogenesis-related factors. *Frontiers in Plant Science* 5: 450. doi:10.3389/fpls.2014.00450.

Petre B, Saunders DGO, Sklenar J, Lorrain C, Win J, Duplessis S, Kamoun S (2015) Candidate effector proteins of the rust pathogen *Melampsora larici-populina* target diverse plant cell compartments. *Molecular Plant-Microbe Interactions* 28:689-700. doi: 10.1094/MPMI-01-15-0003-R.

Petre B, Lorrain C, Saunders DGO, Win J, Sklenar J, Duplessis S, Kamoun S (2015) Rust fungal effectors mimic host transit peptides to translocate into chloroplasts. *Cellular Microbiology* 18:453-465. doi: 10.1111/cmi.12530.

Persoons A, Halkett F, Duplessis S, De Mita S. Demographic and genomic consequences of a major event of adaptation in the pathogenic fungus *Melampsora larici-populina*. *Manuscript in preparation*.

Book chapters

Aime MC, McTaggart AR, Mondo SJ, Duplessis S (2017) Phylogenetics and phylogenomics of rust fungi. *Advances in Genetics* Vol. 99: *In Press*.

Duplessis S, Bakkeren G, Joly, DL, eds. (2016) Genomics Research on Non-model Plant Pathogens: Delivering Novel Insights into Rust Fungus Biology. Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-814-6.

Duplessis S, Bakkeren G, Hamelin R (2014) Advancing knowledge on biology of rust fungi through genomics. *Advances in Botanical Research* 70:173-209.

Presentations

Antoine Persoons. Identification d'effecteurs de Melampsora larici-populina par une approche originale: de la génomique des populations à la génomique fonctionnelle. Présentation orale lors des Rencontres de Phytopathologie-Mycologie de la SFP, Journées Jean Chauvaugeon à Aussois (Janvier 2014).

Persoons A, Morin E, Delaruelle C, Payen T, Halkett F, Frey P, De Mita S, Duplessis S. Patterns of genomic variation in the poplar rust fungus Melampsora larici-populina identify pathogenesis-related factors. Présentation orale lors de l'INRA Nancy and MPI Marburg interdepartmental workshop on plant-fungus interactions, INRA Nancy (Novembre 2014).

Persoons A, Halkett F, De Mita S, Duplessis S. Identification of candidate effectors in the poplar rust fungus Melampsora larici-populina through a population genomics approach. Présentation orale lors de la 25th Fungal Genetics Conference à Asilomar, Etats-Unis (Mars 2015).

Duplessis S. Melampsora larici-populina genomics. Présentation orale lors du Fungal Genomics Workshop du Joint Genome Institute à Walnut Creek, Etats-Unis (Mars 2015).

Duplessis S. The poplar-poplar rust interaction: it's all about perception and manipulation! Séminaire invité de la SFR de Biologie Intégrative et Ecologie, INRA Villenave d'Ornon (Septembre 2015).

Duplessis S. The poplar-poplar rust interaction: it's all about perception and manipulation! Présentation orale lors de l'INUPRAG: INRA/UPSC/CRAG meeting on Plant Integrative Biology, Nancy, France (Octobre 2015).

De Mita S, Halkett F, Frey P, Duplessis S. Update on Melampsora larici-populina genomics. Présentation orale lors des 11èmes Rencontres de Phytopathologie-Mycologie de la Société Française de Phytopathologie, JJC2016, Aussois (Janvier 2016).

De Mita S, Halkett F, Frey P, Duplessis S. Update on Melampsora larici-populina genomics. Poster présenté lors 13th European Conference on Fungal Genetics, ECFG13, Paris (Avril 2016).

Others

Persoons A. Les contournements de résistance par Melampsora larici-populina, l'agent de la rouille du peuplier : impact démographique et déterminisme génétique. Thèse de Doctorat en Biologie Végétale et Forestière de l'Université de Lorraine (soutenue en décembre 2015).