**Plant-Microbe Interfaces: Functional characterization of *Populus* proteins involved in plant-microbe interactions**

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**Project Goals: The goal of the PMI SFA is to characterize and interpret the physical, molecular, and chemical interfaces between plants and microbes and determine their functional roles in biological and environmental systems. *Populus* and its associated microbial community serve as the experimental system for understanding the dynamic exchange of energy, information, and materials across this interface and its expression as functional properties at diverse spatial and temporal scales. To achieve this goal, we focus on 1) defining the bidirectional progression of molecular and cellular events involved in selecting and maintaining specific, mutualistic *Populus*-microbe interfaces, 2) defining the chemical environment and molecular signals that influence community structure and function, and 3) understanding the dynamic relationship and extrinsic stressors that shape microbiome composition and affect host performance.**

Plants, including woody species, live in a microbe-rich environment under natural conditions, and interact with a diverse range of microbes. The outcome of plant-microbe interactions spans the full spectrum from beneficial to pathogenic. With a focus on *Populus* – an ecologically and industrially important tree species whose genome encodes hundreds of small proteins with unknown functions (Yang et al., 2011), we characterized plant proteins involved in interactions with beneficial and pathogenic microbes, respectively, with the goal of pushing the outcomes toward enhanced beneficial impact on the plant host. One aspect of our project focuses on examining the role of *P. trichocarpa* small secreted proteins (PtSSPs) in mutualistic symbiosis. By screening of 417 putative PtSSP-encoding genes that have been shown to be responsive to the mutualistic fungal symbiont *Laccaria bicolor* (Plett et al., 2017), we identified a total of 14 PtSSPs with DNA-binding ability. Focusing on two of the 14 PtSSPs, we performed yeast two hybrid (Y2H) experiments to identify their protein targets in *L. bicolor*. Meanwhile, we performed *Agrobacterium rhizogenes* mediated hairy root transformation and *Agrobacterium tumefaciens* mediated stable transformation, respectively, to overexpress individual PtSSP encoding genes fused to variants of the green fluorescent protein (GFP) gene in the hybrid poplar clone INRA 717-1B4 (*P. tremula* × *P. alba*). We are currently screening for transgenic poplar events and/or poplar roots showing high GFP expression to set up plant- *L. bicolor* co-culture experiments to examine the secretion and movement of the PtSSPs.

In addition, extensive transcriptome changes are often observed in response to inoculation with fungi and bacteria, and efficient manipulation of plant-microbe interactions likely requires the ability to modify the expression of multiple plant genes simultaneously. Therefore, another focus of our project is to develop a multiplex gene regulation system by employing CRISPR activation (CRISPRa), CRISPR interference (CRISPRi), RNA interference (RNAi), and their combinations. Using *P. deltoides* WV94 – a species naturally resistant to the invasive fungal pathogen *Sphaerulina musiva*, we are testing the efficiency of CRISPRa by targeting the orthologs of the G-type lectin receptor-like protein kinase encoding gene *Potri.005G018000*, which confers susceptibility to *S. musiva* (Muchero et al., 2018). We are also developing a protoplast-based system to facilitate the screening of efficient single guide RNAs (sgRNAs) and their combinations.


1. Yang X, Tschaplinski TJ, Hurst GB, Jawdy S, Abraham PE, Lankford PK, et al. (2011). Discovery and annotation of small proteins using genomics, proteomics, and computational approaches. *Genome Research*, 21(4), 634-641.
2. Plett JM, Yin H, Mewalal R, Hu R, Li T, Ranjan P, et al. (2017). *Populus trichocarpa* encodes small, effector-like secreted proteins that are highly induced during mutualistic symbiosis. *Scientific Reports*, 7(1), 1-13.
3. Muchero W, Sondreli KL, Chen JG, Urbanowicz BR, Zhang J, Singan V, et al. (2018). Association mapping, transcriptomics, and transient expression identify candidate genes mediating plant–pathogen interactions in a tree. *Proceedings of the National Academy of Sciences*, 115(45), 11573-11578.



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