Plant-Microbe Interfaces: Functional characterization of small secreted proteins in poplar in relation to P*opulus-Laccaria* symbiosis

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Project Goals: The goal of the Plant-Microbe Interfaces (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.

Symbiosis is a mutually beneficial interaction between two or more organisms. Many fungal lineages have evolved elaborate protein-based signals to influence their hosts in order to support their metabolic requirements during symbiosis (Martin and Kamoun, 2011). Poplar (i.e., *Populus* species) plants are fast-growing trees with great potential for biofuel production and ecosystems service (Yang et al., 2009). Mycorrhizal colonization of poplar roots by ectomycorrhizal fungus *Laccaria bicolor* has a positive impact on the overall tree health and growth. A deep understanding of the molecular mechanism underlying poplar-*Laccaria* interaction will have important implications for increasing bioenergy and ecosystem sustainability.

Recently, we performed RNA sequencing of *P. trichocarpa* roots in symbiosis with *L. bicolor* and identified 417 plant-encoded putative small secreted proteins (SSPs) that were significantly regulated during this poplar-*Laccaria* interaction and four of the five poplar SSPs tested in an *in-vitro* feeding experiment can enter *L. bicolor* nuclei (Plett et al., 2017). Based on this preliminary work, the present study investigates the function of poplar SSPs using both computational and experimental approaches, with a focus on the candidate poplar SSPs that have potential of regulating gene expression in the fungus. Specifically, through computational analysis of DNA-binding potential and secretion signals in the 417 poplar SSPs, we predicted 14 secreted DNA-binding SSPs. Furthermore, for gain-of-function analysis, the poplar genes encoding 12 of these secreted DNA-binding SSPs were over-expressed in multiple plant species (*i.e.*, poplar, *Arabidopsis*, tobacco) through *Agrobacterium*-mediated plant stable transformation. Also, for loss-of-function analysis, two poplar SSP genes were knocked-out using CRISPR/Cas9

technology (Liu et al., 2016). Our preliminary analysis demonstrated that the over-expression in poplar of one of these SSPs (PtSSP1), greatly increased the symbiosis between poplar and *L. bicolor*, along with the PtSSP1 protein moving from the transgenic plants into the nuclei of fungal hyphae. The transgenic poplar, *Arabidopsis* and tobacco plants will be characterized in various aspects such as protein movement from plant to fungal cells, regulation of fungal gene expression, and effect of over-expression/knockout on symbiosis. In summary, we are identifying and characterizing candidate poplar SSPs that could potentially regulate fungal gene expression to promote symbiosis.

References

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