

## 9. Plant-Microbe Interfaces: Discovery of Small Secreted Proteins in *Populus* in Response to Symbiotic Fungus *Laccaria bicolor*

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**Project Goals: The goal of the Plant-Microbe Interfaces SFA is to gain a deeper understanding of the diversity and functioning of mutually beneficial interactions between plants and microbes in the rhizosphere. The plant-microbe interface is the boundary across which a plant senses, interacts with, and may alter its associated biotic and abiotic environments. Understanding the exchange of energy, information, and materials across the plant-microbe interface at diverse spatial and temporal scales is our ultimate objective. Our ongoing efforts focus on characterizing and interpreting such interfaces using systems comprising plants and microbes, in particular the poplar tree (*Populus*) and its microbial community in the context of favorable plant microbe interactions. We seek to define the relationships among these organisms in natural settings, dissect the molecular signals and gene- level responses of the organisms using natural and model systems, and interpret this information using advanced computational tools. PMI research is multidisciplinary by design and multi-institutional in composition.**

Ectomycorrhizal (ECM) symbiosis is a mutualistic association between the roots of many plant species and fungal partners found in soil. Mycorrhizal symbiosis offers various benefits including 1) increasing nutrient availability, 2) improving water use efficiency, 3) enhancing carbon sequestration in terrestrial ecosystems and 4) remediating degraded soils. All of these beneficial aspects make tree-mycorrhizal association an excellent strategy for improving the sustainability of woody crop production. *Populus* is an important woody crop that has been developed for pulp and paper manufacturing, phytoremediation, carbon sequestration, and biofuels production. The soil fungus *Laccaria bicolor* is able to form symbiotic associations with many temperate forest trees including *Populus*. The reference genome sequences along with rich genetic and genomic resources are available for both *Populus* and *Laccaria*. Therefore, the *Populus-Laccaria* interaction is an excellent model system for studying mycorrhizal symbiosis. Understanding the molecular mechanisms underlying the *Populus-Laccaria* interaction would provide potential solutions to protecting and maximizing the value of forest ecosystems, which may lead to novel breeding targets, new sustainable silviculture strategies and better utilization of woody tree species in both industrial and ecological settings.

Small proteins in plants play important regulatory roles in various biological processes such as stress response, flowering, and hormone signaling. However, our knowledge about plant small proteins in relation to mycorrhizal symbiosis is very limited. To address this limitation, we performed genome-wide analysis of *Populus* small proteins, with a focus on small secreted proteins, in response to *L. bicolor* inoculation. Based on RNA- seq data analysis, we identified 1,242 computationally predicted

*Populus* small proteins (SmPs) that were up-/down-regulated in response to *Laccaria* inoculation. In the small protein set, 417 proteins (33.6%) were predicted to be small secreted proteins (SSP). Gene ontology analysis revealed that some SmPs were involved in biological processes relevant to plant-microbe interactions, such as response to fungus, jasmonic acid metabolism, salicylic acid-mediated signaling pathway, and cell-to-cell communication.

*P. trichocarpa* is much more heavily colonized by *L. bicolor* than *P. deltoides*. Analysis of *Populus* genome resequencing data revealed several *Populus* SmP genes present in *P. trichocarpa* genotypes (“93-968” and “Nisqually-1”) but absent in *P. deltoides* genotypes (“D124” and “ILL-101”), suggesting that these SmP genes contribute to the genotypic difference in *Populus-Laccaria* interaction. Importantly, we tested the 39 predicted SSPs using yeast trap system and 33% of them were confirmed to be secretory proteins. The high-confidence SSP candidate genes are being characterized using molecular and genetic approaches. This research generated new knowledge about the molecular basis of *Populus-Laccaria* symbiosis.

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