Plant-Microbe Interfaces: Genome-wide identification of *Populus* small proteins responsive to mycorrhizal symbiosis

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Project Goal: Understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between plants and microbes. *Populus* and its associated microbial community serves as an initial experimental system for understanding how these molecular events manifest themselves within the spatially, structurally, and temporally complex scales of natural systems. To achieve this goal, we will focus on 1) characterizing the natural variation in *Populus* microbial communities within complex environments, 2) elucidating *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships, and 3) performing metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

The mycorrhizal symbiosis, representing the most widespread plant-microbe association, offers various benefits including 1) enhancing carbon sequestration in terrestrial ecosystems, 2) increasing nutrient availability, 3) remediating degraded soils and 4) improving water use efficiency. All of these beneficial aspects make plant-mycorrhizal association an excellent strategy for improving the sustainability of bioenergy feedstock production. However, our knowledge about the molecular mechanism underlying mycorrhizal symbiosis is still very limited. To address this limitation, we performed a genome-wide analysis of *Populus* genes in response to *Laccaria* inoculation (Fig. 1). We identified 1,282 transcripts differentially expressed during mycorrhizal development, among which 764 novel transcripts have not been documented in the current version of *Populus* genome annotation. About 11% of the 1,282 differentially-expressed transcripts encode small proteins of <100 amino acids in length. Gene ontology analysis revealed that mycorrhizal symbiosis between *Populus* and *Laccaria* involves different sets of genes over the time course of symbiosis development (Table 1). A large number of protein sequences encoded by these transcripts were predicted to be located in the nuclei, suggesting that they may play roles in gene expression regulation. Interestingly, many protein sequences were predicted to be located in both nuclei and extracellular space, implying that they are putative signaling proteins responsible for communication between *Populus* and *Laccaria.* Also, we found that 11 transcripts were up-regulated through the time course of mycorrhizal development, suggesting that they are important to the maintenance of plant-fungus interaction. Since *P. trichocarpa* better associates with *Laccaria* when compared to *P. deltoides*, we examined the differences in the DNA-seq coverage of differentially expressed genes (based on genome resequencing data) between the two *Populus* species. We identified 10 genes that are present in *P. trichocarpa* but absent in *P. deltoides*. Lastly, we performed homology search in 24 other sequenced plant genomes ranging from algae to moss to angiosperm and found that only about 40% of the *Laccaria*-responsive genes in *Populus* have homologs in other plant species, suggesting that lineage-specific molecular mechanism may play an important role in regulating plant response to mycorrhizal fungi.

Populus root samples collected at 2, 4, and 12 weeks after *Laccaria* innoculation

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Transcriptome sequencing

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Identification of transcripts up-/down-regulated by *Laccaria*

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Functional prediction
Protein domains; sub-cellular localization; GO annotations, etc.

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Experimental validation

Figure 1. A pipeline for discovery of *Populus* small proteins mediating plant-fungus interaction.

Table 1: Biological processes enriched in up-/down-regulated *Populus* genes at 2, 4, and 12 weeks after *Laccaria* inoculation.

	2 weeks	4 weeks	12 weeks
Up regulation	Response to stimulus	Macromolecule metabolic process	Nitrogen compound metabolic process
Down regulation	Nitrogen compound metabolic process	Nitrogen compound metabolic process	Biosynthetic process

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