Plant-Microbe Interfaces: Small-RNA (sRNA) and open reading frame (sORF) response to endo- and ecto-mycorrhizal symbioses in *Populus*

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Project Goals: The goal of the PMI SFA is to 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 the 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 focus on 1) characterizing host and environmental drivers for diversity and function in the *Populus* microbiome, 2) utilizing microbial model system studies to elucidate *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships and 3) develop metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

Mycorrhizal fungi are a diverse group of beneficial symbionts that colonize the roots of more than 90% of higher plant species. In doing so, these fungi play an important role in the maintenance of the plant health by promoting water cycling, facilitating nutrient exchange, and protection from a variety of biotic and abiotic stresses; and in exchange, the fungus receives plant-fixed carbon (Bonfante and Genre 2010; Smith and Read 2008). Mycorrhizal fungi can be classified as fungi colonizing the intercellular spaces of the roots (ectomycorrhizas, ECM) e.g., *Laccaria bicolor* or developing within the root cells (endomycorrhizas/arbuscular mycorrhiza fungi, AMF) e.g., *Rhizophagus irregularis* (formerly *Glomus intraradices*) (Bonfante and Genre 2010). The genetic contribution from the plant and fungi for the establishment and maintenance of this mutualistic symbiosis is somewhat unclear. A number of studies support the hypothesis that fungi-derived protein signals, or effectors, largely facilitate the symbiotic interaction. In this regard, the genome of *L. bicolor* revealed a large number of small secreted proteins (SSPs), many of which are expressed and accumulated in the fungal hyphae colonizing root tissue, potentially facilitating symbiosis (Martin et al. 2008).

Plant colonization by fungi is highly specific and requires that the plant distinguishes between beneficial and pathogenic fungi, and ensures that the relationship remains advantageous.

Therefore, it may be naive to believe that the plant remain a silent partner in a beneficial symbiotic interaction and this notion was challenged by several studies (el Zahar Haichar et al. 2014). In the current study, we characterized the small RNA response to AMF and ECM fungi. We performed small RNA-sequencing of roots from *P. deltoides* infected with *R. irregularis* and *L. bicolor*, and *P. trichocarpa* infected with *R. irregularis* and *L. bicolor*. Roots of mock uninfected were used as a control. We found significantly differentially expressed transcripts between the treatments and control (either upregulated or downregulated), of which some are novel transcripts aligning to non-genic regions of *P. trichocarpa.* Since sRNA can potentially encode sORF (Li et al. 2014; Ruiz-Orera et al. 2014), we used Open Reading Frame (ORF) Finder (Wheeler et al. 2003) to scan the significantly differentially expressed sRNAs for potential sORF. In total, we could predict several sORFs in the differentially expressed transcripts from the treatments in *P. deltoides* and *P. trichocarpa* respectively. Also, we analyzed the small RNA sequencing datasets to identify microRNAs (miRNAs) genes that were responsive to mycorrhiza treatments. Our analysis identified 369 putative miRNAs, of which 239 were classified as 'plant novel' miRNAs and134 showed up-/down-regulated expression in response to fungal inoculation. Furthermore, 157 miRNA-target pairs were revealed through degradome analysis.

Our results suggest that the response from the plants are in fact far more complex than previously thought and that sRNA may be an important factor underlying plant-microbe symbiotic interaction.

References

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