Transgenic Poplar Lines Reveal Host Genes Involved in Defense Against Rust

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Project Goals: The objective of this research is to investigate the molecular basis for the virulence of *Melampsora larici-populina* towards *Populus* spp. in order to address the formidable challenge of engineering durable resistance in poplar against leaf rust. The research plan builds on dual host systems and known host defense proteins and pathogen avirulent effectors along with genome-wide approaches to identify key pathogenesis effectors of *Melampsora larici-populina* that target poplar defense and nutrient acquisition. The overarching hypothesis of the project is that rust effectors with homology to other fungal effectors will either bypass the poplar immune system and/or suppress effector-triggered immunity through conserved mechanisms, and that an analysis of these interaction networks will provide for new approaches to develop rust-resistant poplar. The project goals are to mine key rust effectors that suppress host immunity, elucidate interaction networks in poplar targeted by key rust effectors, and to generate transgenic poplar resources for identification of genes involved in pathogen infection or host defense triggered by rust. Our ongoing work on the molecular mechanisms involved in pathogen recognition by the plant innate immune system, the physiological processes that haustorium-dependent pathogens use to commandeer nutrient acquisition systems from the host, are enabling us to construct novel poplar biotypes for use as bioenergy crops. This work is providing new resources to the scientific community to expand the potential for disease-resistant poplar as a bioenergy feedstock.

Abstract: *Melampsora larici-populina* contains a large number of genes that encode candidate secreted effector proteins (CSEPs), which are thought to play significant roles in promoting rust infection in *Populus* spp. CSEPs genes encode small, cysteine-rich secreted proteins that are specifically up-regulated for expression during infection, signifying a key role in host colonization by the pathogen. Two groups of effectors have been the target of this project: (1) 160 *Melampsora larici-populina* CSEPs belonging to 67 structural families, all of which share significant homology among pathogenic rust, *Septoria* or powdery mildew fungi; and (2) a unique 117-member family, whose members show virtually no similarity to any protein in sequence databases. A rapid, quantitative screen was developed to assess *Melampsora* CSEPs that affect poplar immunity. Attenuation (or enhancement) of salicylate levels stemming from changes in HR was measured quantitatively using LC-MS to assess the impact of *Melampsora larici-populina* CSEPs on mounting an immune response. Transient expression of CSEPs along with HR-promoting effectors in tobacco leaves, in poplar leaves, and in poplar protoplasts identified two effects on host immunity: many CSEPs suppress the host immune response, unexpectedly, several promote an immune reaction, triggering a hypersensitive response (HR). In an effort to characterize HR in poplar, pathogenesis related (PR) gene expression was

analyzed in poplar treated with salicylic acid, in transgenic poplar lines expressing HR-promoting *R* genes, and in transgenic poplar lines expressing *Melampsora* CSEPs. Biochemical analysis reveals that pathogenic effectors promote a burst of reactive oxygen species (ROS) that correlates with PR1 gene expression. These lines are being used to identify additional host genes involved in defense against rust, and to evaluate susceptibility mechanisms in compromised hosts.

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