

Transgenic Poplar Lines to Probe Host Genes Involved in Defense Against Rust

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Project Goals: *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. A screen has been developed in tobacco leaves and poplar protoplasts for CSEPs that affect host immunity. Two subsets of effectors have been targeted for analysis: one with significant homology to CSEPs in other fungal pathogens of plants, and another, large family, containing unique members with no similarity to any proteins in sequence databases. Although results from these screens show that many CSEPs suppress the host immune response, unexpectedly, several promote an immune reaction, triggering a hypersensitive response. Genes for these immune-reactive effectors have been used to construct stable transgenic poplar lines with constitutive and inducible promoters to identify host genes involved in defense against rust, for the evaluation of susceptibility mechanisms in compromised hosts, and to provide reagents to the community.

Abstract: Fungal pathogens of plants, such as *Melampsora larici-populina* which promotes rust disease in *Populus* spp., contain genes for small, cysteine-rich secreted proteins that are specifically up-regulated for expression during infection, signifying a key role in host colonization by the pathogen. Computational analysis of the *Melampsora larici-populina* genome suggests that its complement of 1,524 candidate secreted effector proteins (CSEPs) belong to 807 structural families, including 600 single-member families, another 206 families that contain 2-36 members, and one family that has undergone a remarkable expansion to 117 members. 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. The two subsets of CSEPs were expressed transiently in tobacco along with well-known 'autoactive' domains of *R* genes or an *R* gene-*AvrP* pair that promotes a hypersensitive response (HR) or HR-like cell death. 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. Although roughly half of the effectors screened affect host immunity, interestingly, a smaller subset stimulates an immune response in the screen, and when examined alone, promote a hypersensitive response in tobacco. Transient expression of the reactive CSEPs in poplar protoplasts is challenging, and low throughput, but initial

results support the tobacco-based large-scale screens. A high-efficiency transformation protocol has been developed and used to construct stable transgenic poplar lines that express *Melampsora* CSEPs from strong, constitutive or inducible promoters. These lines are being used to identify host genes involved in defense against rust, for the evaluation of susceptibility mechanisms in compromised hosts, and to provide reagents to the community.

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