

The role of beneficial microbes in stress management of *Populus*

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain. CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Populus is an important commercial crop and one of the leading candidates for bioenergy production; however, the commercial use of *Populus* is easily impacted by abiotic and biotic stresses such as drought, nitrogen limitation as well as major pathogens (e.g. rust fungi, *Melampsora*). In order to maximize sustainable *Populus* biomass production, we proposed to engineer microbial community to alleviate stresses and nutrient deficiency limiting growth and productivity using native microbial collection. We assembled a fungal-bacterial community that benefit each other and are symbionts of *Populus*. At field scale, we demonstrated these beneficial microbes are stable in soil and *Populus* roots (after two years) while they increase plant yield of 30%. We also observed that this microbial treatment significantly reduces the severity of drought and infection of rust pathogens (*Melampsora spp.*).

As a consequence of this result, we further investigated the effect of the beneficial microbes during drought and *Melampsora* infection in *Populus*. There is little known on the role of beneficial root microbes in the modulation of drought in poplar and in poplar immune system to decrease susceptibility to leaf pathogens. We performed greenhouse experiments under well-watered and drought conditions and identified how beneficial microbes enhanced plant tolerance under water stress. In parallel, we employ closed microcosm system with beneficial microbial inoculants and sterile *Populus* plant treated with or without the rust pathogen. Thus, coupling transcriptomic and metabolomic approaches, we aim to characterize the defense-related metabolites and genes primed in poplar by beneficial microbes and to identify specific-defense microbial metabolite produced or transferred to the plant partner. This pioneer work contributes to the understanding of the molecular mechanisms to alleviate impactful stresses in woody plants during beneficial associations to further exploit them and develop a sustainable bioenergy feedstock.

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