Creation of an Acyltransferase Toolbox for Plant Biomass Engineering

Craig A. Bingman,[1] **Brian G. Fox**[2*] (bgfox@wisc.edu),[1] Steven D. Karlen,[1] Shawn D. Mansfield,[2] Kirk Vander Meulen,[1] John Ralph,[1] Rebecca A. Smith[1]

[1]University of Wisconsin-Madison, Madison, Wisconsin USA; [2]University of British Columbia, Vancouver, British Columbia CANADA

Project Goals and Abstract:

The goal of this project is to expand the understanding and utility of the **acyl-CoA ligases** and **BAHD acyltransferases** in plant engineering. The impressive diversity of bioproducts created by their coordinated action includes simple aromatic and aliphatic esters, derivatives of flavanols, anthocyanidins, alkaloids, terpenoids, cutin, suberin, and many others. Our work has shown that monolignol esters can be polymerized into plant lignins with essentially no impact on growth and viability, but with profound positive impacts on the processing and energy content of the engineered plant. The ester-enriched lignin created by engineering efforts using monolignol ferulates, biosynthesized via hydroxycinnamoyl-CoA monolignol transferases, to produce so-called 'zip-lignin' is particularly encouraging.

Overall there is little experimental evidence for the breadth of substrate specificities, rates of catalysis, structural properties, stabilities, tissue localization, or *in planta* synergistic capabilities of the acyl-CoA ligases and BAHD acyltransferases, particularly for important bioenergy crops such as poplar, sorghum, and switchgrass. As such, these limited understandings represent major knowledge gaps, and potential bottlenecks to the successful engineering of new properties into plant cell walls and optimization of the biosynthesis of many other useful metabolic products.

We are proceeding with a systematic evaluation of all members of these important gene families in DOE's priority bioenergy plants. The proposed research adopts an integrative pipeline approach, including genomics, bioinformatics, cell-free translation, *in vitro* biochemistry, structural biology, optical and fluorescence microscopy, chemical synthesis, state-of-the-art methods for cell wall analysis including NMR spectroscopy, and cell-wall analytics. We will leverage in-depth knowledge of plant cell wall structure, lignin biosynthesis, plant transformation and cultivation, and practical aspects of processing plant material. Results will show how we have used this experimental pipeline to identify new acyltransferases with a breadth of new activities and have begun to introduce them into bioenergy plants and assess their impacts.

The major deliverable of this project will be experimentally validated lists of acyltransferase and ligase genes from DOE priority bioenergy plants with an accounting of their *in planta* abilities to modify the properties of bioenergy crop plants. Ultimately, our acyltransferase toolbox will provide an experimentally validated *'knock-out/knock-in'* list for performing precision engineering of plant metabolism to obtain new and improved properties.

This work is funded by the US Department of Energy, Department of Science, Office of Biological and Environmental Research, Genome-Enabled Plant Biology for Determination of Gene Function Funding Opportunity Number DE-FOA-00002060, project number 248370.