Genetic engineering to produce C-lignin deposition in plant stems

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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.

Catechyl (C) lignin, discovered by our team in 2012 in the seed coats of diverse non-crop species [1,2], holds promise as a high-value co-product in biorefining [3,4] due to its more linear polymer structure. A CBI goal is to understand the biosynthetic pathway to C-lignin and develop strategies to introduce the polymer into cell walls of CBI target species (poplar and switchgrass) through genetic engineering without compromising plant growth performance. Lignin biosynthesis switches from formation of classical guaiacyl (G) lignin to C-lignin during development of the seed coat of the plant *Cleome hassleriana*. We are using molecular genetics to better understand C-lignin biosynthesis in Cleome seeds [5] and construct this pathway in vascular tissues of bioenergy crops. Our results suggest that: i) the transcriptional repression of monolignol *O*-methyltransferases coupled with expression of specific forms of cinnamyl alcohol dehydrogenase and laccase underlie the switch to C-lignin, ii) young seedlings of *Medicago truncatula* naturally produce C-lignin and its level was doubled by co-disruption of the genes COMT and CCoAOMT, and iii) C-lignin accumulation is limited by both the provision of the caffeyl alcohol monomer and the initiation of its polymerization by specific laccases. Future work with transgene constructs and isotopic experiments with labeled caffeyl alcohol have been designed to test the role of specific laccases on C-lignin biosynthesis.

References

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