Down-Regulation of the Caffeic acid *O*-methyltransferase Gene in Switchgrass Reveals a Novel Monolignol Analog

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves 1) designing plant cell walls for rapid deconstruction and 2) developing multitalented microbes for converting plant biomass into biofuels in a single step (consolidated bioprocessing). Addressing the roadblock of biomass recalcitrance will require a greater understanding of plant cell walls from synthesis to deconstruction. This understanding would generate models, theories and finally processes that will be used to understand and overcome biomass recalcitrance. This grand challenge calls for an integrated research approach, which is illustrated by the project described below. BESC research is multidisciplinary by design and multi-institutional in composition.

Down-regulation of the caffeic acid 3-O-methyltransferase (COMT) gene in the lignin biosynthetic pathway of switchgrass (*Panicum virgatum*) resulted in cell walls of transgenic plants releasing more constituent sugars after pretreatment by dilute acid and treatment with glycosyl hydrolases from an added enzyme preparation and from *Clostridium thermocellum*. Fermentation of both wild-type and transgenic switchgrass after milder hot water pretreatment with no water washing showed that only the transgenic switchgrass inhibited *C. thermocellum*. Gas chromatography-mass spectrometry-based metabolomics were undertaken on cell wall aqueous extracts to determine the nature of the microbial inhibitors. Metabolomic analyses of the transgenic biomass revealed the presence of a novel monolignol-like metabolite, identified as *trans*-3, 4-dimethoxy-5-hydroxycinnamyl alcohol (*iso*-sinapyl alcohol). Down-regulated COMT SWG accumulated *iso*-sinapyl alcohol, its glucoside *iso*-syringin, and putative lignan conjugates. *iso*-sinapyl alcohol is likely synthesized from its acid and aldehyde precursors that are also only evident in COMT-deficient plants. As hypothesized, COMT-deficient plants have lowered concentrations of sinapyl alcohol and increased concentrations of phenolic acid and aldehyde inhibitors of microbial fermentation. Quantum chemical calculations were used to predict the most likely homodimeric lignans generated from dehydration reactions, but these products were not evident in plant samples. Such analyses indicated fewer and different conjugation sites for iso-sinapyl alcohol than for sinapyl alcohol, but dehydrogenation polymerization assays indicated that *iso*-sinapyl alcohol does not affect the size of the lignin polymer. Although there was no indication that *iso*-sinapyl alcohol was integrated into the cell wall, diversion of substrates from sinapyl alcohol to free *iso*-sinapyl alcohol, its glucoside, and associated upstream lignin pathway changes, including increased phenolic aldehydes and acids, are associated with more facile cell wall deconstruction, and to the observed inhibitory effect on microbial growth. The release of wall constituents by mild pretreatment hydrolysis may be an informative approach that integrates wall-polymerized and associated metabolic responses that occurred over time rather than at a single point in time.

[The BioEnergy Science Center is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.]