Lignin Management: Optimizing Yield in Lignin-Modified Plants

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Abstract

This project aims to maximize the utility of plant lignocellulosic biomass as an abundant, sustainable, and carbon-neutral energy feedstock by optimizing both its yield and composition to facilitate downstream conversions to fuel and electricity. Working independently with different lignin-deficient mutants, the partners have discovered novel genes that mitigate the growth defects [so-called lignin-modification-induced dwarfism (LMID)] seen in severely lignin-depleted plants. Revealing the mechanism(s) by which this mitigation occurs is critical to fundamental understanding and useful manipulation of how plants partition carbon and may enable biomass manipulation for carbon sequestration in the future. We have undertaken several projects to determine the causes of, and to reduce the effects of, LMID. By expressing the lignin biosynthetic gene *CSE* only in vessel elements, the effects of LMID in *cse* mutants is lessened. We are also utilizing mutant screens in lignin biosynthetic mutants to discover novel genes involved in LMID. One screen, in the *ccr1* background, has led to the discovery of a mutant that partially restores the growth defect of the original line, yet maintains saccharification efficiency. The gene responsible for this trait has been identified, and tests are underway to understand the mechanism behind the LMID reduction. Another mutant screen, in the highly dwarfed *ref8* (*c3h*) background, has identified more than 20 lines that suppress LMID,

designated as *growth inhibition relieved (gir)*. One of these, *GIR1*, has been identified through a mapping by next generation sequencing approach as a β-importin important for translocation of a transcription factor responsible for regulation of lignin biosynthesis genes. Another screen for suppressors of a mutant in the transcriptional complex Mediator, which controls the LMID response in *ref8* plants, has yielded a variety of mutants which could lead to a greater understanding of how LMID is induced in *c3h* plants.

Towards identifying the pathways responsible for LMID, a metabolomic pipeline has been established to identify metabolites altered in lignin mutants. The detection and authentication of compounds identified by this pipeline will be enhanced because we have synthesized several lignin trimers and tetramers, allowing us to generate metabolite profiles for them. We have also pioneered the production of monolignols that can be selectively tagged after incorporation into lignin, allowing us to probe lignin structure to a greater degree. Several phenylpropanoid metabolites were screened for their ability to affect *Arabidopsis* growth, and one was found to alter growth. The incorporation of alternative monolignols has also been investigated, as the inclusion of hydroxycinnamaldehydes leads to an increase in saccharification potential. We are also targeting the orthologs of high saccharification mutants previously identified in *Arabidopsis* for implementation in energy crops (barley, poplar). The new CRISPR/Cas9 technology will allow for targeted knock-outs of lignin biosynthesis genes in barley. This will allow for greater effects on plant lignin content and composition.

Publications

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