

Towards New Degradable Lignin Types

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Abstract

Lignin is the major limiting factor in the conversion of lignocellulosic biomass into liquid biofuels. Over the past decade, researchers have made a number of exciting observations that have significantly extended our possibilities of engineering plant cell walls. One of these findings is that plants can tolerate large variations in the composition of the normal lignin units in the cell wall. Striking examples were the high levels of hydroxycinnamaldehydes into lignin in plants deficient in CAD, and the enormous shifts in S/G composition in plants with modified F5H expression. Second, it was shown that plants tolerate the incorporation of pathway intermediates that are normally not found in lignin, such as 5-hydroxyconiferyl alcohol, again apparently without adverse effects on plant health. Third, detailed analysis of lignin structure in transgenic and wild type plants revealed the presence of lignin units other than the classical three monolignols. For example, we showed that sinapyl *p*-hydroxybenzoate and ferulate are to be considered authentic monomers as they are cross-coupled into the polymer [1-3]. Fourth, there are now a number of studies indicating that wood quality can be improved by altering lignin composition to the benefit of chemical pulping and saccharification [4-6]. Unpublished data from our lab show that CCR-deficient poplars release ~ two-fold higher amounts of glucose upon saccharification of dried stems, and a range of Arabidopsis mutants with defects in lignin biosynthesis genes improve glucose yield up to 4 fold (the Boerjan lab, unpublished data). The observation that lignin composition is malleable without necessarily affecting plant growth and development opens interesting perspectives. Indeed, exporting phenolic molecules that can couple with normal monolignols into the apoplastic space may give a polymer with improved properties in terms of degradability. One recent key publication already demonstrates the concept. Feeding corn cell suspensions with coniferyl ferulate (one of the proposed target molecules) results in lignified cell walls that can be delignified at lower temperatures [7].

The long term goal of the project is to identify natural products (called ‘target molecules’) that can be biosynthesized in energy crops, translocated through the plasma membrane and cross-coupled with lignin units such that the final lignin polymer is more susceptible to chemical cleavage, or is more hydrophilic, or is less cross-linked with hemicelluloses. Ideally, the structures of the target molecules are very similar to traditional monolignols so that they can be exported to the wall using the same transport system.

The project is divided into four tasks. Task 1 aims at defining which molecules are good targets for engineering and targeting to plant cell walls. We have compiled a list of target molecules. Task 2 aims at cloning biosynthetic genes for target molecules and overexpressing these in bioenergy crops. We have identified an Arabidopsis gene that, when over-expressed, is expected to steer the flux through the phenylpropanoid pathway into the biosynthesis of one of the candidate molecules. We are currently generating transgenic Arabidopsis to support our hypothesis. Task 3 is more challenging, and aims at rerouting a selected number of target molecules, of which the biosynthetic pathway and subcellular localization are already partially known, to the cell wall in transgenic plants. Because several of the proposed target molecules are located in the vacuole, we have identified a putative transporter that is involved in translocation/retention of several target molecules into the vacuole. Transgenic plants silencing this transporter are being generated. Task 4 is the most risky, and aims at identifying biosynthetic pathways for promising target molecules by a combination of genetics and metabolomics. To this end, we have metabolically profiled Arabidopsis plants and identified several of our target molecules in this model system, opening perspectives to clone their biosynthetic pathways using genetic strategies.

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