An association genetics approach to identifying novel targets for lignin biosynthesis engineering and improved biofuel feedstocks

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The ‘problem’ of lignin

Lignin provides plants with structural support, protection from pathogens and waterproofing for the vascular system. It’s most familiar as the defining component of wood. However, these very same properties mean that lignin also makes the utilization of cellulose for industrial purposes difficult. Lignin is tightly associated with cellulose in the cell wall, and must be degraded in order to get to the cellulose, which can then be used to make bioethanol, or paper, for example. The degradation incurs an enormous energy input penalty for industrial processing. In the case of biofuels, this is especially problematic as it makes the energy balance of the resulting fuel less favorable.

Alternate monomer strategy

There are two basic strategies for solving the ‘problem’ of lignin - one is to engineer a plant with less lignin, and the other is to engineer a plant with a different kind of lignin that is easier to break down. This project is about the second strategy. Lignin is generally described as being made up of three kinds of phenylpropanoid monomer, however mass spectrometry techniques developed recently in our lab have found many more kinds of units than previously thought. This reveals exciting opportunities to engineer the polymer by increasing the incorporation of these units, and so potentially altering the polymer’s degradability without affecting its functionality in the plant.

Screening Arabidopsis tissue library for compounds known within the plant kingdom

Arabidopsis is a plant that provides a good model for lignification despite the fact that it is a herbaceous plant. It also has the advantage of large numbers of genetic tools and resources available for identifying targets for metabolic engineering, and their implementation.

In the first instance we screened a large library of Arabidopsis tissues and ecotypes for ~60 alternative lignin monomers, that are known to be synthesized within the plant kingdom – as the majority of metabolites in any one plant, including Arabidopsis, are unknown. This provided a handful of ‘hits’, and a second target list was drawn up of ~80 further molecules which is in the process of being screened.

Results: we found feruloyl hexose, sinapoyl hexose, ferulic acid hexose, p-coumaric acid malic acid, feruloyl malic acid, sinapoyl malic acid, dopamine, glucose, and ferulic acid from our first list. Plus, mass spectra, suggestive of acetate isomers and cimiracemates B and D which may be confirmed by NMR.

Candidate monomers

Four different classes of molecules have been defined whose incorporation into lignin is predicted to provide degradability improvements.

Searching for hydroxycinnamic acid conjugated compounds

Hydroxycinnamic acids (HCA’s, left) are phenylpropanoid pathway intermediates closely related to the monolignols. They can incorporate into lignin and often form compounds with ester bonds, like those of Class III

Results:

To enrich our results for genes proximate to the metabolic conversions we are most interested in, rather than using absolute metabolite levels, we are using the ratio of putative substrates and products, to represent a measure of the conversion rate of a particular enzyme. Our preliminary analysis tested 15 ratios. This lead to 5 associations, 3 of which were deemed to be feasible ‘hits’ according to the known gene function, as illustrated below; we are now in the process of analysing GWAS data for both the stem and rosette metabolome in Arabidopsis.

Genome Wide Association Study (GWAS)

The purpose of a GWAS is to provide an indication of which genes might be responsible for variation in a particular trait, such as the amount of a particular metabolite. This provides us with targets for genetic metabolic engineering.

Ingredients for a GWAS:

A population of plants that vary genetically; we are using a population of 250 different ecotypes from all over the world.

Defined genetic variation: this is done by genotyping the plants by microarray hybridization

Then the variation in a trait must be measured. Our traits consist of metabolite levels or ratios between putative substrates and products.

A statistical analysis is then done to test for associations between the genotype at any particular locus and the quantitative value of the phenotype.

This allows us to select gene candidates for the biosynthesis of alternative lignin monomers and lignin pathway intermediates.

Future perspectives:

The identification of any new genes involved in lignin metabolism and related biosynthetic pathways is important in the identification of novel targets for the engineering of lignin, for the purpose of making plants that are more efficiently converted into biofuel. Our dataset now puts us in a prime position to find such targets.