Novel Mutants Optimized for Lignin, Growth and Biofuel Production via Re-Mutagenesis

Investigators
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Objective
To identify novel combinations of genes that can be manipulated in order to improve the yield of simple sugars (saccharification) from plant cell walls during biofuel production.

Background
The bulk of plant biomass is composed of plant cell walls. Significant improvements in the efficiency of biomass conversion into bioenergy or biofuels can be envisaged by breeding plant varieties with optimum cell wall properties using conventional or genetic modification approaches. In nature, the hydrophobic phenolic polymer, lignin, specifically reduces permeability and porosity in plant cell walls to prevent biological and chemical attack. However, during bioethanol production, plant materials must be pretreated to remove lignin before hydrolysis of cellulose (and fermentation of released sugars to yield alcohol) can proceed, adding significantly to the cost and energy consumption of the process. Significant cost savings and improved energy balance could be achieved by reducing the amount of lignin in plants or by tailoring its structure to enable more efficient cellulose hydrolysis.

Approach
Plants with modified lignin will be mutagenized to make secondary genetic changes that may further enhance the plant material for cellulosic saccharification and bioethanol production. A high-throughput saccharification screen will be used to quickly identify plants with beneficial modifications (Figure 1). This will be followed by full characterization of the plants and cloning of the disrupted gene(s). Currently, the only plant species in which this program can be achieved in a three-year timeframe is the model plant, Arabidopsis, where the small genome size allows mutagenesis screens to be performed at a manageable scale and where the identification of genes disrupted by mutation is routine. However, proof-of-concept in Arabidopsis can be rapidly followed by cloning and manipulation of the orthologous genes in biofuel crops.

Plants with mutations that affect lignin content or structure have already been identified and been shown to perform differently on extraction with pretreatments. Applying non-targeted secondary mutagenesis to existing mutants will allow the identification of second-site modifiers that either enhance or suppress (alleviate) the primary phenotype. Examples of such possible modifiers could be genes that act redundantly with the primary mutation, genes that act in parallel pathways, genes that encode factors that interact physically with the mutant gene product, or genes on pathways that become activated or repressed by a second-site suppressor. The beauty and power of these modifier screens are that they are unbiased and are therefore likely to lead to truly new insights into how genes and pathways interconnect to enhance or suppress a given phenotype.
There is huge potential and value in applying this strategy to lignin manipulation. It represents an approach to "stacking" cell wall changes beneficial for biofuel production, and also a way of identifying genes involved in, or connected to, lignification. Despite more than a decade of intense research in the area, there is still much to learn about this process and how it can be modified to our benefit.

Figure 1: Schematic of method for identifying mutants with improved sugar release on enzymatic digest. EMS (Ethane methyl sulfonate) mutagenesis of seeds causes alterations in genes leading to altered traits in plants progeny (M1 and M2) compared to the original plants.