

Lab breakthrough can lead to cheaper biofuels, improved crops, and new products from plants

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Henrik Scheller (left) and Dominique Loque hold a tray of *Arabidopsis Thaliana* plants, which they used in their research. Credit: Berkeley Lab photo

(Phys.org) —Imagine being able to precisely control specific tissues of a plant to enhance desired traits without affecting the plant's overall function. Thus a rubber tree could be manipulated to produce more natural latex. Trees grown for wood could be made with higher lignin

content, making for stronger yet lighter-weight lumber. Crops could be altered so that only the leaves and certain other tissues had more wax, thus enhancing the plant's drought tolerance, while its roots and other functions were unaffected.

By manipulating a plant's [metabolic pathways](#), two scientists at the Department of Energy's Lawrence Berkeley National Laboratory (Berkeley Lab), Henrik Scheller and Dominique Loqué, have figured out a way to genetically rewire plants to allow for an exceptionally high level of control over the spatial pattern of gene expression, while at the same time boosting expression to very high levels. Now they have launched a startup company called Afingen to apply this technology for developing low-cost biofuels that could be cost-competitive with gasoline and corn ethanol.

"With this tool we seem to have found a way to control very specifically what tissue or cell type expresses whatever we want to express," said Scheller. "It's a new way that people haven't thought about to increase metabolic pathways. It could be for making more cell wall, for increasing the stress tolerance response in a specific tissue. We think there are many different applications."

Cost-competitive biofuels

Afingen was awarded a Small Business Innovation Research (SBIR) grant earlier this year for \$1.72 million to engineer switchgrass plants that will contain 20 percent more fermentable sugar and 40 percent less lignin in selected structures. The grant was provided under a new SBIR program at DOE that combines an SBIR grant with an option to license a specific technology produced at a national laboratory or university through DOE-supported research.

"Techno-economic modeling done at (the Joint BioEnergy Institute, or

JBEI) has shown that you would get a 23 percent reduction in the price of the biofuel with just a 20 percent reduction in lignin," said Loqué. "If we could also increase the sugar content and make it easier to extract, that would reduce the price even further. But of course it also depends on the downstream efficiency."

Scheller and Loqué are plant biologists with the Department of Energy's Joint BioEnergy Institute (JBEI), a Berkeley Lab-led research center established in 2007 to pursue breakthroughs in the production of cellulosic biofuels. Scheller heads the Feedstocks Division and Loqué leads the cell wall engineering group.

The problem with too much lignin in biofuel feedstocks is that it is difficult and expensive to break down; reducing lignin content would allow the carbohydrates to be released and converted into fuels much more cost-effectively. Although low-lignin plants have been engineered, they grow poorly because important tissues lack the strength and structural integrity provided by the lignin. With Afingen's technique, the plant can be manipulated to retain high lignin levels only in its water-carrying vascular cells, where [cell-wall](#) strength is needed for survival, but low levels throughout the rest of the plant.

The centerpiece of Afingen's technology is an "artificial positive feedback loop," or APFL. The concept targets master transcription factors, which are molecules that regulate the expression of genes involved in certain biosynthetic processes, that is, whether certain genes are turned "on" or "off." The APFL technology is a breakthrough in plant biotechnology, and Loqué and Scheller recently received an R&D 100 Award for the invention.

An APFL is a segment of artificially produced DNA coded with instructions to make additional copies of a master transcription factor; when it is inserted at the start of a chosen biosynthetic pathway—such as

the pathway that produces cellulose in fiber tissues—the plant cell will synthesize the cellulose and also make a copy of the master transcription factor that launched the cycle in the first place. Thus the cycle starts all over again, boosting cellulose production.

The process differs from classical genetic engineering. "Some people distinguish between 'transgenic' and 'cisgenic.' We're using only pieces of DNA that are already in that plant and just rearranging them in a new way," said Scheller. "We're not bringing in foreign DNA."

Other licensees and applications

This breakthrough technique can also be used in fungi and for a wide variety of uses in plants, for example, to increase food crop yields or to boost production of highly specialized molecules used by the pharmaceutical and chemical industries. "It could also increase the quality of forage crops, such as hay fed to cows, by increasing the sugar content or improving the digestibility," Loqué said.

Another intriguing application is for biomanufacturing. By engineering plants to grow entirely new pharmaceuticals, specialty chemicals, or polymer materials, the plant essentially becomes a "factory." "We're interested in using the plant itself as a host for production," Scheller said. "Just like you can upregulate pathways in plants that make cell walls or oil, you can also upregulate pathways that make other compounds or properties of interest."

Separately, two other companies are using the APFL technology. Tire manufacturer Bridgestone has a cooperative research and development agreement (CRADA) with JBEI to develop more productive rubber-producing plants. FuturaGene, a Brazilian paper and biomass company, has licensed the technology for exclusive use with eucalyptus trees and several other crops; APFL can enhance or develop traits to optimize

wood quality for pulping and bioenergy applications.

"The inventors/founders of Afingen made the decision to not compete for a license in fields of use that were of interest to other companies that had approached JBEI. This allowed JBEI to move the technology forward more quickly on several fronts," said Robin Johnston, Berkeley Lab's Acting Deputy Chief Technology Transfer Officer. "APFL is a very insightful platform technology, and I think only a fraction of the applications have even been considered yet."

Afingen currently has one employee—Ai Oikawa, a former postdoctoral researcher and now the director of plant engineering—and will be hiring three more in November. It is the third startup company to spin out of JBEI. The first two were Lygos, which uses synthetic biology tools to produce chemical compounds, and TeselaGen, which makes tools for DNA synthesis and cloning.

Provided by Lawrence Berkeley National Laboratory

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