Scientists identify key genes for increasing oil content in plant leaves

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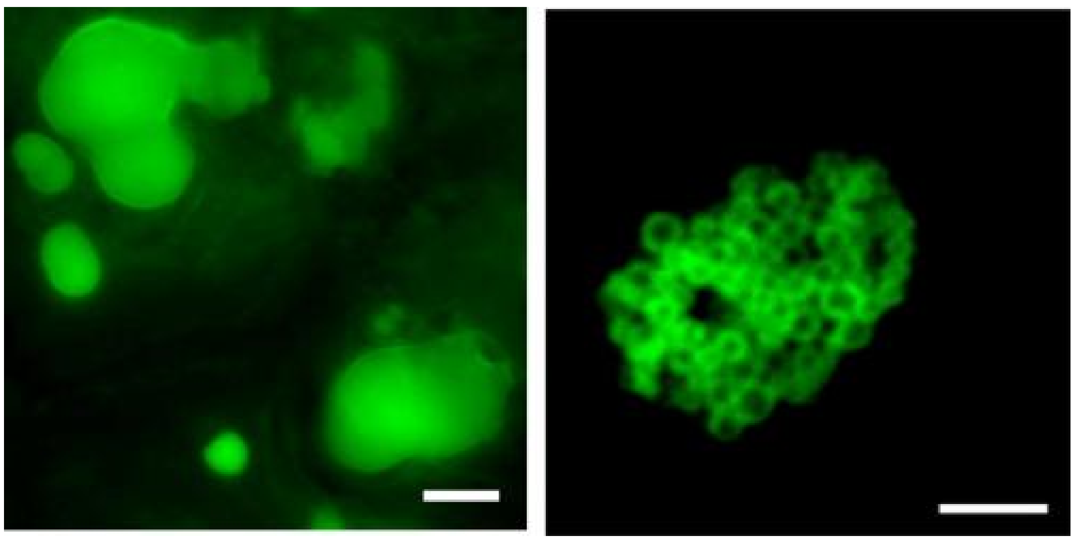

Increasing oil accumulation in leaves: Overexpressing the gene for PDAT, an enzyme involved in oil production, caused plant leaves to accumulate large amounts of oil in large globules (left). When the scientists also added a gene for olesin, a protein known to encapsulate oil droplets (fused to green fluorescent protein to confirm its location), clusters of smaller, more stable oil droplets formed (right). Credit: Brookhaven National Laboratory

(Phys.org) —Scientists at the U.S. Department of Energy's Brookhaven National Laboratory have identified the key genes required for oil production and accumulation in plant leaves and other vegetative plant tissues. Enhancing expression of these genes resulted in vastly increased oil content in leaves, the most abundant sources of plant biomass-a finding that could have important implications for increasing the energy content of plant-based foods and renewable biofuel feedstocks. The research is described in two new publications in *The Plant Journal* and *Plant Cell*.

"If we can transfer this strategy to crop plants being used to generate renewable energy or to feed livestock, it would significantly increase their energy content and nutritional values," said Brookhaven biochemist Changcheng Xu, who led the research. The experiments were carried out in large part by Xu's group members Jilian Fan and Chengshi Yan.

Think about it in the familiar terms of calories: Oil is twice as energy-dense as carbohydrates, which make up the bulk of leaves, stems, and other vegetative plant matter. "If you want to cut calories from your diet, you cut fat and oils. Conversely, if you want to increase the caloric output of your biofuel or feed for livestock, you want more oil," said Xu.

But plants don't normally store much oil in their leaves and other vegetative tissues. In nature, oil storage is the job of seeds, where the energy-dense compounds provide nourishment for developing plant embryos. The idea behind Xu's studies was to find a way to "reprogram" plants to store oil in their more abundant forms of biomass.

The first step was to identify the genes responsible for oil production in vegetative plant tissues. Though oil isn't stored in these tissues, almost all plant cells have the capacity to make oil. But until these studies, the pathway for oil biosynthesis in leaves was unknown.

"Many people assumed it was similar to what happens in seeds, but we tried to look also at different genes and enzymes," said Xu.

Unraveling the genes

The scientists used a series of genetic tricks to test the effects of overexpressing or disabling genes that enable cells to make certain enzymes involved in oil production. Pumping up the factors that normally increase oil production in seeds had no effect on oil production in leaves, and one of these, when overexpressed in leaves, caused growth and developmental problems in the plants. Altering the expression of a different oil-producing enzyme, however, had dramatic effects on leaf oil production.

"If you knock out (disable) the gene for an enzyme known as PDAT, it doesn't affect oil synthesis in seeds or cause any problems to plants, but it dramatically decreases oil production and accumulation in leaves," Xu said. In contrast, overexpressing the gene for PDAT-that is, getting cells to make more of this enzyme-resulted in a 60-fold increase in leaf oil production.

An important observation was that the excess oil did not mix with cellular membrane lipids, but was found in oil droplets within the leaf cells. These droplets were somewhat similar to those found in seeds, only much, much larger. "It was as if many small oil droplets like those found in seeds had fused together to form huge globules," Xu said.

Bigger droplets may seem better, but they're not, explained Xu. Oil in these oversized droplets is easily broken down by other enzymes in the cells. In seeds, he said, oil droplets are coated with a protein called oleosin, which prevents the droplets from fusing together, keeping them smaller while also protecting the oil inside. What would happen in leaves, the scientists wondered, if they activated the gene for oleosin along with PDAT?

The result: Overexpression of the two genes together resulted in a 130-fold increase in production of leaf oil compared with control plants. This time the oil accumulated in large clusters of tiny oleosin-coated oil droplets.

Identifying the mechanism

Next the scientists used radio-labeled carbon (C-14) to decipher the biochemical mechanism by which PDAT increases oil production. They traced the uptake of C-14-labeled acetate into fatty acids, the building blocks of membrane lipids and oils. These studies showed that PDAT drastically increased the rate at which these fatty acids were made.

Then the scientists decided to test the effects of overexpressing the newly identified oil-increasing genes (PDAT and oleosin) in a variant of test plants that already had an elevated rate of fatty acid synthesis. In this case, the genetic boost resulted in even greater oil production and accumulation-170-fold compared with control plants-to the point where oil accounted for nearly 10 percent of the leaf's dry weight.

"That potentially equals almost twice the oil yield, by weight, that you can get from canola seeds, which right now is one of the highest oil-yielding crops used for food and biodiesel production," said Xu. Burning plant biomass with such energy density to generate electricity would release 30 to 40 percent more energy, and the nutritional value of feed made from such energy-dense biomass would also be greatly enhanced.

"These studies were done in laboratory plants, so we still need to see if this strategy would work in bioenergy or feed crops," said Xu. "And there are challenges in finding ways to extract oil from leaves so it can be converted to biofuels. But our research provides a very promising path to improving the use of plants as a source of feed and feedstocks for producing renewable energy," he said.

Xu is now collaborating with Brookhaven biochemist John Shanklin to explore the potential effect of overexpressing these key genes on oil production in dedicated biomass crops such as sugarcane.

More information: *Plant Cell* paper: Dual Role for Phospholipid:Diacylglycerol Acyltransferase: Enhancing Fatty Acid Synthesis and Diverting Fatty Acids from Membrane Lipids to Triacylglycerol in Arabidopsis Leaves, www.plantcell.org/content/earl … .113.117358.abstract

The Plant Journal paper: Phospholipid:diacylglycerol acyltransferase-mediated triacylglycerol biosynthesis is crucial for protection against fatty acid-induced cell death in growing tissues of Arabido, onlinelibrary.wiley.com/doi/10 … 1/tpj.12343/abstract

Provided by Brookhaven National Laboratory

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