Creating plants that make their own fertilizer

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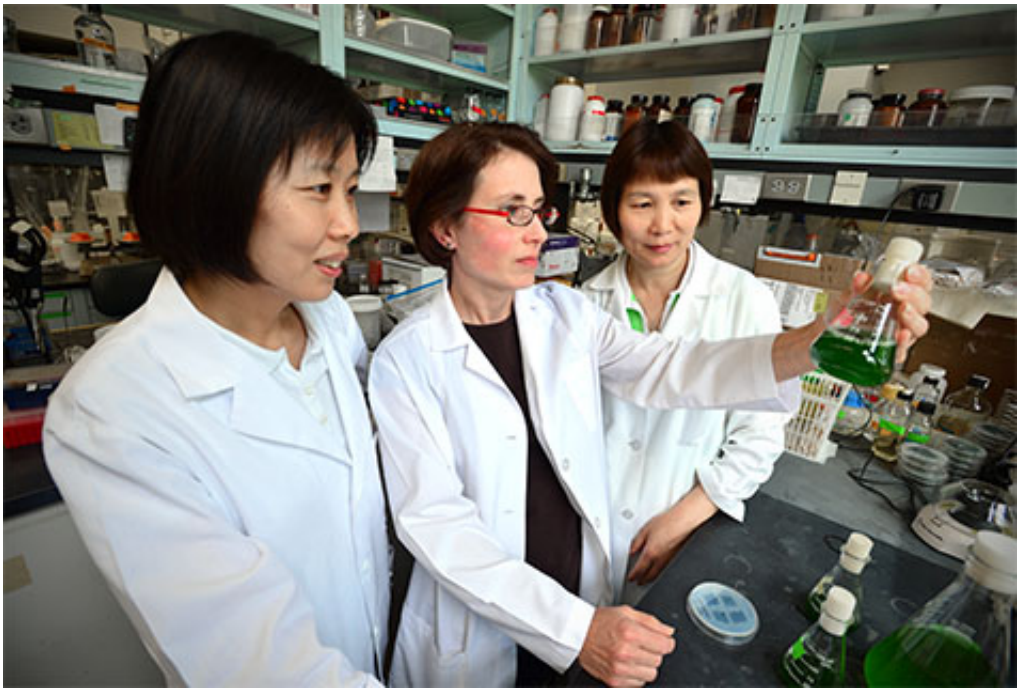

Nancy Duan (left), Michelle Liberton and Lingxia Zhao are members of a team that has taken the first proof-of-principle steps toward inserting the genes needed to fix nitrogen — otherwise found only in bacteria and the bacteria-like Archae — into the cells of crop plants. Credit: JAMES BYARD/WUSTL

Scientists at Washington University are undertaking an ambitious project to engineer tiny nitrogen-fixing devices within photosynthetic cells.

Since the dawn of agriculture, people have exercised great ingenuity to pump more nitrogen into crop fields. Farmers have planted legumes and plowed the entire crop under, strewn night soil or manure on the fields,

shipped in bat dung from islands in the Pacific or saltpeter from Chilean mines and plowed in glistening granules of synthetic fertilizer made in chemical plants.

No wonder Himadri Pakrasi's team is excited by the project they are undertaking. If they succeed, the chemical apparatus for nitrogen fixation will be miniaturized, automated and relocated within the plant so nitrogen is available when and where it is needed—and only then and there.

"That would really revolutionize agriculture," said Pakrasi, PhD, the Myron and Sonya Glassberg/Albert and Blanche Greensfelder Distinguished University Professor, in Arts & Sciences, and director of the International Center for Advanced Renewable Energy and Sustainability (I-CARES) at Washington University in St. Louis.

Engineering with biological parts

Although there is plenty of nitrogen in the atmosphere, atmospheric nitrogen is not in a form plants can use. Atmospheric nitrogen must be "fixed," or converted into compounds that make the nitrogen available to plants.

Much of modern agriculture relies on biologically available nitrogenous compounds made by an industrial process, developed by German chemist Fritz Haber in 1909. The importance of the Haber-Bosch process, as it eventually was called, can hardly be overstated; today, the fertilizer it produces allows us to feed a population roughly a third larger than the planet could sustain without synthetic fertilizer.

On the other hand, the Haber-Bosch process is energy-intensive, and the reactive nitrogen released into the atmosphere and water as runoff from agricultural fields causes a host of problems, including respiratory

illness, cancer and cardiac disease.

Pakrasi thinks it should be possible to design a better nitrogen-fixing system. His idea is to put the apparatus for fixing nitrogen into plant cells, the same cells that hold the apparatus for capturing the energy in sunlight.

The National Science Foundation just awarded Pakrasi and his team more than $3.87 million to explore this idea further. The grant will be administered out of I-CARES, a university-wide center that supports collaborative research regionally, nationally, and internationally in the areas of energy, the environment and sustainability.

This award is one of four funded by the National Science Foundation jointly with awards funded by the Biotechnology and Biological Sciences Research Council in the United Kingdom. The teams will collaborate with one another and meet regularly to share progress and successes. The NSF release is available here.

A proof of principle

As a proof of principle, Pakrasi and his colleagues plan to develop the synthetic biology tools needed to excise the nitrogen fixation system in one species of cyanobacterium (a phylum of green bacteria formerly considered to be algae) and paste it into a second cyanobacterium that does not fix nitrogen.

The team includes: Tae Seok Moon, PhD, and Fuzhong Zhang, PhD, both assistant professors of energy, environmental and chemical engineering in the School of Engineering & Applied Science at Washington University; and Costas D. Maranas, the Donald B. Broughton Professor of Chemical Engineering at Pennsylvania State University.

"Ultimately what we want to do is take this entire nitrogen-fixation apparatus—which evolved once and only once—and put it in plants," Pakrasi said. "Because of the energy requirements of nitrogen fixation, we want to put it in chloroplasts, because that's where the energy-storing ATP molecules are produced." In effect, the goal is to convert all crop plants, not just the legumes, into nitrogen fixers.

Amazing cycling chemistry

All cyanobacteria photosynthesize, storing the energy of sunlight temporarily in ATP molecules and eventually in carbon-based molecules, but only some of them fix nitrogen. Studies of the evolutionary history of 49 strains of cyanobacteria suggest that their common ancestor was capable of fixing nitrogen and that this ability was then repeatedly lost over the course of evolution.

The big hurdle to redesigning nitrogen fixation, however, is that photosynthesis and nitrogen fixation are incompatible processes. Photosynthesis produces oxygen as a byproduct and oxygen is toxic to nitrogenase, the enzyme needed to fix nitrogen. This is why most organisms that fix nitrogen work in an anaerobic (oxygenless) environment.

Cyanobacteria that both photosynthesize and fix nitrogen separate the two activities either in space or in time. Cyanothece 51142, a cyanobacterium Pakrasi's lab has studied for more than 10 years, does it through timing.

Cyanothece 51142 has a biological clock that allows it to photosynthesize during the day and fix nitrogen at night. During the day, the cells photosynthesize as fast as they can, storing the carbon molecules they create in granules. Then, during the night, they burn the carbon molecules as fast as they can. This uses up all the oxygen in the

cell, creating the anaerobic conditions needed for nitrogen fixation.

Thus, the environment within the cell oscillates daily between the aerobic conditions needed for capturing the energy in sunlight and the anaerobic conditions needed for fixing nitrogen.

A single mega transfer

The scientists have chosen their proof-of-principle project very carefully to maximize the odds it will work.

Cyanothece 51142 is particularly attractive as a parts source for the project because it has the largest contiguous cluster of genes related to nitrogen fixation of any cyanobacterium. Roughly 30 genes are part of the same functional unit under the control of a single operating signal, or promoter.

The scientists hope this cluster of genes can be moved to another cyanobacterial strain in a single mega-transfer. The one they've picked as the host, Synechocystis 6803, is the best-studied strain of cyanobacteria. Not only has its genome been sequenced, it is naturally "transformable" and able to integrate foreign DNA into its genome by swapping it with similar native strands of DNA.

But it's actually the next step in the project that will provide the greater challenge for Pakrasi and his team. The scientists will need to figure out how to connect the transplanted nitrogen-fixing gene cluster to Synechocystis' clock. "Like every cyanobacterium," Pakrasi said, " Synechocystis has a diurnal rhythm. But how to tap into that rhythm we don't know yet. We have some ideas we're going to test, but that's where the challenge lies."

Overcoming the challenge of sustainably producing food for a world

population of more than 7 billion while reducing pollution and greenhouse gases will require more than luck. Odds are it will take a daring, "out of the box" idea like this one.

Provided by Washington University in St. Louis