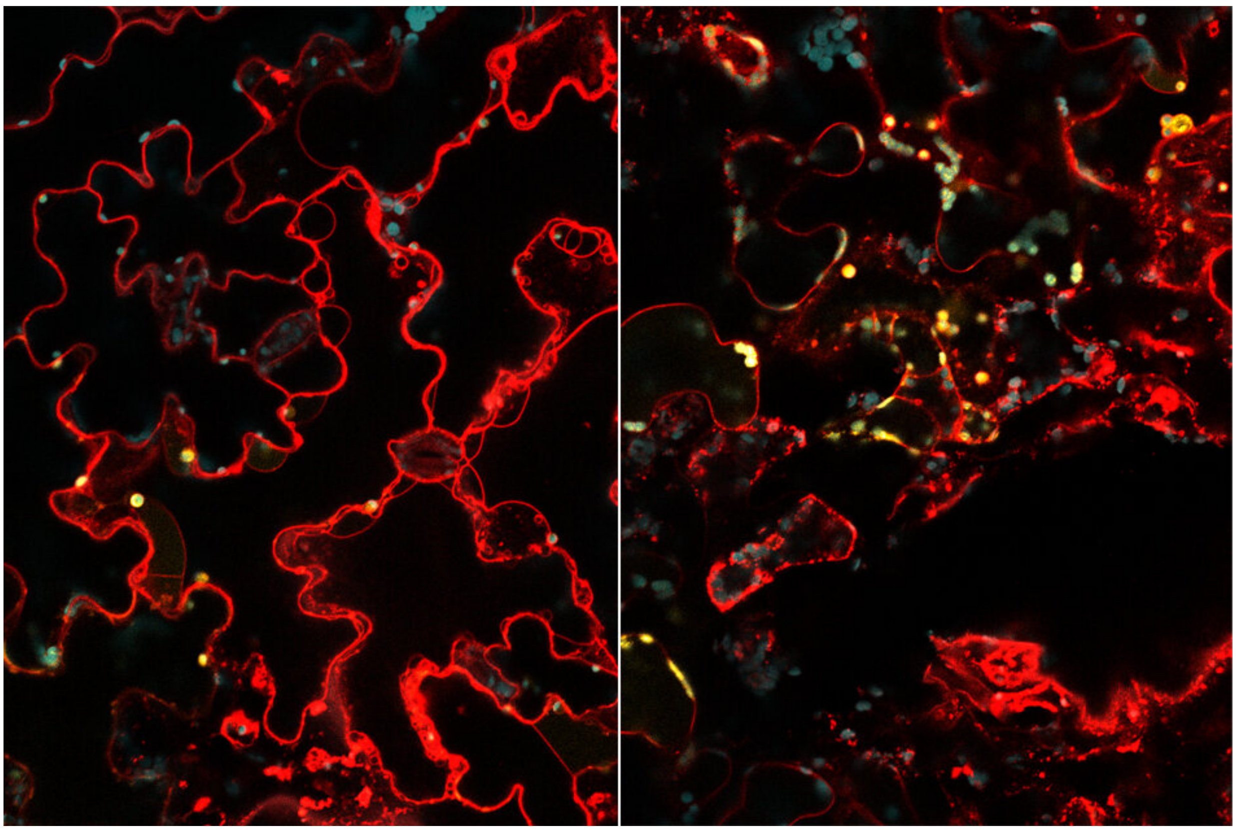
Team uses nanoparticles to deliver genes into plant chloroplasts

February 25 2019



MIT researchers have developed a genetic tool that could make it easier to engineer plants that can survive drought or resist fungal infections. Their technique, which uses nanoparticles to deliver genes into the chloroplasts of plant cells, works with many different plant species. Credit: Massachusetts Institute of Technology

MIT researchers have developed a new genetic tool that could make it easier to engineer plants that can survive drought or resist fungal infections. Their technique, which uses nanoparticles to deliver genes into the chloroplasts of plant cells, works with many different plant species, including spinach and other vegetables.

This new strategy could help plant biologists to overcome the difficulties involved in genetically modifying plants, which is now a complex, time-consuming process that has to be customized to the specific plant species that is being altered.

"This is a universal mechanism that works across plant species," says Michael Strano, the Carbon P. Dubbs Professor of Chemical Engineering at MIT, about the new method.

Strano and Nam-Hai Chua, the deputy chair of the Temasek Life Sciences Laboratory at the National University of Singapore and a professor emeritus at Rockefeller University, are the senior authors of the study, which appears in the Feb. 25 issue of *Nature Nanotechnology*.

"This is an important first step toward chloroplast transformation," Chua says. "This technique can be used for rapid screening of candidate genes for chloroplast expression in a wide variety of crop plants."

This study is the first to emerge from the recently launched Singapore-MIT Alliance for Research and Technology (SMART) program in Disruptive and Sustainable Technologies for Agricultural Precision (DiSTAP), which is headed by Strano and Chua. The lead authors of the study are former MIT postdoc Seon-Yeong Kwak, who is now the scientific director of the DiSTAP program, and MIT graduate student Tedrick Thomas Salim Lew.

Targeting chloroplasts

A few years ago, Strano and his colleagues discovered that by tuning the size and electrical charge of nanoparticles, they could design the nanoparticles to penetrate plant cell membranes. This mechanism, called lipid exchange envelope penetration (LEEP), allowed them to create plants that glow, by embedding nanoparticles carrying luciferase, a light-emitting protein, into their leaves.

As soon as the MIT team reported using LEEP to get nanoparticles into plants, plant biologists began asking if it could be used to genetically engineer plants, and more specifically, to get genes into chloroplasts. Plant cells have dozens of chloroplasts, so inducing the chloroplasts (instead of just the nucleus) to express genes could be a way to generate much greater quantities of a desired protein.

"Bringing genetic tools to different parts of the plant is something that plant biologists are very interested in," Strano says. "Every time I give a talk to a plant biology community, they ask if you could use this technique to deliver genes to the chloroplast."

The chloroplast, best known as the site of photosynthesis, contains about 80 genes, which code for proteins required to perform photosynthesis. The chloroplast also has its own ribosomes, allowing it to assemble proteins within the chloroplast. Until now, it has been very difficult for scientists to get genes into the chloroplast: The only existing technique requires using a high-pressure "gene gun" to force genes into the cells, which can damage the plant and is not very efficient.

Using their new strategy, the MIT team created nanoparticles consisting of carbon nanotubes wrapped in chitosan, a naturally occurring sugar. DNA, which is negatively charged, binds loosely to the positively charged carbon nanotubes. To get the nanoparticles into plant leaves, the researchers apply a needleless syringe filled with the particle solution to the lower side of the leaf surface. Particles enter the leaf through tiny pores called stomata, which normally control water evaporation.

Once inside the leaf, the nanoparticles pass through the plant cell wall, cell membranes, and then the double membranes of the chloroplast. After the particles get inside the chloroplast, the slightly less acidic environment of the chloroplast causes the DNA to be released from the nanoparticles. Once freed, the DNA can be translated into proteins.

In this study, the researchers delivered a gene for yellow fluorescent protein, allowing them to easily visualize which plant cells expressed the protein. They found that about 47 percent of the plant cells produced the protein, but they believe that could be increased if they could deliver more particles.

More resilient plants

A major advantage of this approach is that it can be used across many plant species. In this study, the researchers tested it in spinach, watercress, tobacco, arugula, and Arabidopsis thaliana, a type of plant commonly used in research. They also showed that the technique is not limited to carbon nanotubes and can potentially be extended to other types of nanomaterials.

The researchers hope that this new tool will allow plant biologists to more easily engineer a variety of desirable traits into vegetables and crops. For example, agricultural researchers in Singapore and elsewhere are interested in creating leafy vegetables and crops that can grow at higher densities, for urban farming. Other possibilities include creating drought-resistant crops; engineering crops such as bananas, citrus, and coffee to be resistant to fungal infections that threaten to wipe them out; and modifying rice so that it doesn't take up arsenic from groundwater.

Because the engineered genes are carried only in the chloroplasts, which are inherited maternally, they can be passed to offspring but can't be transferred to other plant species.

"That's a big advantage, because if the pollen has a genetic modification, it can spread to weeds and you can make weeds that are resistant to herbicides and pesticides. Because the chloroplast is passed on maternally, it's not passed through the pollen and there's a higher level of gene containment," Lew says.

More information: Chloroplast-selective gene delivery and expression in planta using chitosan-complexed single-walled carbon nanotube carriers, *Nature Nanotechnology* (2019). DOI: 10.1038/s41565-019-0375-4 , www.nature.com/articles/s41565-019-0375-4

Provided by Massachusetts Institute of Technology

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