

Q&A: Xiaohan Yang on transforming plants for a cleaner future

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Scientist Xiaohan Yang's research at the Department of Energy's Oak Ridge National Laboratory focuses on transforming plants to make them better sources of renewable energy and carbon storage.



He works with the ORNL-led Center for Bioenergy Innovation, or CBI, a DOE Bioenergy Research Center where scientists are developing feedstock crops like <u>poplar trees</u> that grow quickly, require less water and fertilizer and are easily broken down and converted into sustainable aviation fuels.

What is plant transformation?

Plant transformation occurs when we transfer DNA from one plant to another and create a better hybrid. We identify the gene, or group of <u>genes</u>, linked to a desired physical trait like drought tolerance or higher yield, and then insert them into a different plant. The goal is to trigger the target plant to exhibit the trait we want to see.

The transformation is successful when we make sure the expressed trait is stable, meaning that the trait is inherited by the plant's progenies, generation after generation. At ORNL, we're focused on developing plants that are easy to grow at a low cost and produce a lot of biomass material that can be converted into clean jet fuels and bio-based chemicals.

Why is the research important?

The development and <u>field testing</u> of a genetically stable, transformed plant ready for commercialization can take years. Speeding up that process is essential to help meet today's climate challenges with sustainable jet fuels and bioproducts derived from plants—specifically, nonfood crops that can grow on marginal lands in less than ideal conditions.

That knowledge could even be transferred to other crops, supporting new plants that are resilient to environmental challenges like drought, pests



and diseases, with better yield and quality. If we can get these plants to absorb more carbon from the atmosphere and transfer it into the soil, that also supports decarbonization efforts. So, we end up with carbon sequestration belowground from the <u>root system</u>, and biofuels for jets made from aboveground biomass.

What have been some of your discoveries so far?

We have discovered genes in semi-arid plants that are linked to drought resistance and accelerated growth. Plants like agave have evolved to survive in dry environments by developing a special kind of photosynthesis called CAM [crassulacean acid metabolism]. CAM plants absorb carbon dioxide via leaf pores called stomata and convert it into an organic acid for storage at night when water is less likely to evaporate.

During the day stomata remain closed, conserving water and using sunlight to convert CO_2 to chemical energy. We identified the genes related to CAM through sequencing the RNA and DNA in two different species, Agave americana and Kalanchoe fedtschenkoi.

We also discovered a single variant gene in CAM that triggers two pathways simultaneously in plants: one for carbon fixation and plant growth, and the other spurring production of proline, an amino acid linked to stress tolerance. Tobacco plants engineered with the gene produced more biomass, even under stress. The gene acted as a master regulator, switching on other genes in the plant.

While sequencing the messenger RNA in agave, we also discovered the REVEILLE1 gene that controls when the plant goes dormant and when it begins budding, which can help us extend their growing season. We inserted the gene in poplar and developed a tree that grows taller with larger leaves and thicker stems. Poplar transformed with REVEILLE1 showed a 166% increase in biomass when grown in a greenhouse.



What are you focused on now?

We recently developed and demonstrated a way to transform plants even faster by successfully engineering multiple genes into plants at once in an approach called gene-stacking. We created a split selectable marker system that accelerates transformation using inteins. Inteins are protein segments that have a natural ability to split off from larger proteins, allowing for the re-assembly of the partial fragments into a fully functional protein. The system includes markers that identify the transformed cells, support their stability and make genetic engineering events detectable using light-based biosensors.

The selectable marker system is important. By making the genetic changes visible in ultraviolet light, we can use a UV flashlight to detect whether our transformation was successful.

This avoids the time-consuming and costly process of sampling part of the plant for molecular characterization and speeds up the breeding of new plants. We can track molecular changes in plants in the greenhouse and in the field faster and easier with this visible biomarker, significantly speeding up our phenotyping work that connects plant traits to their underlying genetics.

We demonstrated the simultaneous insertion of four genes in poplar and are now working on stacking 12 genes at once to create a better hybrid. We think the technique can be refined to support the stacking of up to 20 genes. This new approach to plant transformation is one of the most important developments to come out of CBI's biomass feedstock research in the last 15 years.

What's ahead in the field of plant transformation?



One of my midterm goals is related to the biomarker system we've developed, integrating genetic engineering with phenotyping for an accelerated plant genetic engineering ecosystem. The technology enables a noninvasive, low-cost, high-throughput system for phenotyping at multiple levels: the molecular level, the metabolic level and the plant level.

Our light-based biosensor process can replace slow, painstaking phenotyping data collection with a one-pass, real-time detection system to tell us whether we've successfully created engineered plants with desired traits. This has the potential to be a disruptive innovation in plant research, similar to the technology in science fiction movies where you use a no-touch instrument to scan the body and determine a person's state of health.

We will test the application in our Advanced Plant Phenotyping Laboratory at ORNL. We're building a plant transformation pipeline that begins with <u>synthetic biology</u> and connects to accelerated phenotyping.

I have a longer-term goal to conduct research in an exciting new area: plant synthetic genomics. We are approaching this process when we engineer multiple genes into plants. With synthetic genomics, we can design an entirely new chromosome to be added to poplar with all the new traits we want, instead of just modifying existing genes.

The technique has already been demonstrated in yeast, and we hope that we can establish this cutting-edge capability in <u>plants</u> within 10 years. It's similar to buying a 100-year-old house and then trying to modernize it. It is very difficult. Why not build a new house that's designed with everything you want. There's a lot of enthusiasm in the plant science community about the potential of synthetic genomics and how to solve the technological challenges to get there.



Provided by Oak Ridge National Laboratory

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