Improving poplar biomass production under stress conditions

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Poplar provides a fast-growing woody feedstock for biofuels. Credit: Andrea Starr | PNNL

Inside the phytotron, rows of white industrial cabinets hide the life expected in a greenhouse. A peek through a square viewing port, however, reveals the green energy growing inside the bright chamber. These specific poplar saplings may not survive the hardships of prolonged droughts and heat waves, but they are helping a team of researchers make ones that can.

Stresses, such as high temperatures and a lack of freshwater, can cause

reduced crop growth or even complete loss. The rising frequency of these stressful periods inspires a race to find more tolerant plants. But traditional plant breeding methods are slow, requiring trial and error across multiple generations. Now, a collaborative group of researchers is redesigning poplar, a bioenergy crop, based on its specific genome in just a few years.

The growing human population is placing increasing demands on uses of all types of land. For the biofuel industry to compete with conventional fuel sources it must use land resources, but ideally not require the same premium lands used to grow food. If biofuel crops are designed to be more stress tolerant, they can maintain high biomass production on marginal lands—keeping the industry competitive.

Last year, the Department of Energy's Office of Science awarded $5.5 million to improve poplar biomass production to the multi-institutional team of the University of California, Davis, Pacific Northwest National Laboratory, and two other universities: University of Tennessee (UT) and West Virginia University (WVU). The project title is "SyPro Poplar: Improving Poplar Biomass Production under Abiotic Stress Conditions: An Integrated Omics, Bioinformatics, Synthetic Biology, and Genetic Engineering Approach."

For the five-year project, the team is "developing some poplar trees by a transgenic approach that are tolerant to multiple stresses at the same time," says co-principal investigator Amir H. Ahkami. Ahkami is a plant molecular biologist with EMSL, the Environmental Molecular Sciences Laboratory, a DOE Office of Science user facility at PNNL.

Combinations

Past studies have only focused on one abiotic stress—water deficiency, elevated temperature or soil salinity—at a time, explains Ahkami. In

reality, plants can suffer from a combination of stresses simultaneously. So, finding a poplar-specific solution under these circumstances is the project's target.

The trick may be developing a series of synthetic promoters—promoters control gene expression—that turn on the appropriate stress-tolerant genes. But getting there will take a combination of advanced approaches and novel technologies.

The field trials at WVU will tell the research team how the transgenic poplar plants fair under real environmental conditions as opposed to the controlled greenhouse settings. Credit: Stephen DiFazio | WVU

"We are bringing together cell biologists, molecular biologists, physiologists and a national lab in a common effort," says plant biologist Eduardo Blumwald, the lead principal investigator and a distinguished professor of cell biology at UC Davis. "This is a multi-disciplinary approach, and I think that this is the most important element."

Ahkami adds, "And we brought together a really good group of experts

for this project."

Plant transformation requires a DNA sequence that combines a promoter and a gene. Starting with plants that have been placed under stress conditions will help in identifying the important stress-responsive genes and proteins. Blumwald is applying controlled stress tests to poplar plants in a research greenhouse at UC Davis. The treatments include reducing the water availability, lowering the water quality and turning off greenhouse cooling.

On specific dates throughout the treatments, Blumwald samples leaves and roots from the poplar specimens and ships them to PNNL for cell- and tissue-specific omics analysis. Transcriptomics is the study of the complete set of RNA transcripts produced by an organism's genome, while proteomics is the study of its proteins. The cell-type specific omics approach is unique and will be informative.

Using this multiomics approach, Ahkami can identify the highly differentially expressed genes and proteins under stress conditions—which are expressed more or less compared to those under normal conditions. Then, using a genetic engineering tool such as Clustered Regularly Interspaced Short Palindromic Repeats or CRISPR, he can verify the function of a gene of interest.

He can then identify stress-responsive promoters based on the stress-induced genes and proteins and, in collaboration with experts at UT, use bioinformatics to discover a particular regulatory element, or a motif, that guides the engineering of a promoter.

Design-build-test

By engineering their own synthetic promoters, the researchers are not restricted to only those promoters found in nature. They can design ones

that are specific to the species and cell type so that they turn on the right genes only when needed.

These poplar plants from another study illustrate how quickly they can grow in the field after about two years. Credit: Stephen DiFazio | WVU

"We want to interfere the least as possible with the development of the tree," says Blumwald. "If we are going to express a gene constitutively all the time, we run the risk that the gene will make the plants a bit smaller, for example."

He likens it to a person going to the supermarket. If someone goes food shopping on the way to work, then he or she must deal with that food in the car or on the bus and at work—it's cumbersome, he says. By shopping after work, that person gets food only when food is needed. The stress resistant traits should only be expressed when wanted.

Using the knowledge gained from omics and existing promoter libraries, the researchers will design, build and test a suite of potential synthetic

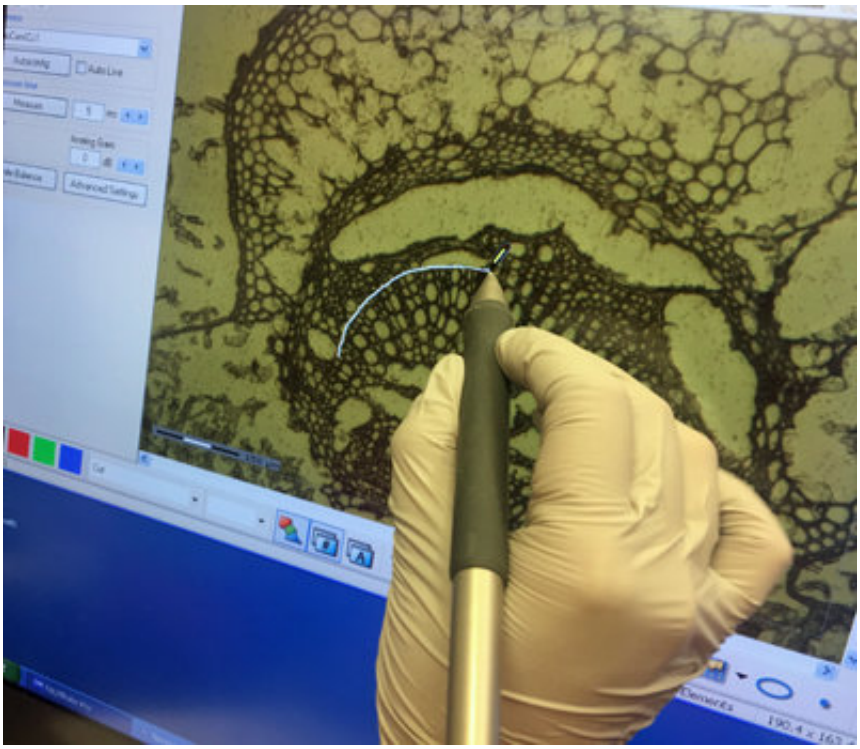
promoters. More than a hundred promoters will be screened with poplar leaf- and root-derived protoplasts—cells with removed cell walls—using a robotic system in co-principal investigator C. Neal Stewart, Jr.'s laboratory at UT. To know if a promoter is working, the researchers will look for a fluorescent protein to light up, explains Stewart, a professor of plant sciences, who focuses on plant genetics.

"It's one of the things where you get a lot of failures, but all you need is one or two hits," says Stewart, "And then it's a success."

The team will install the best artificial promoters into poplar plants to drive the gene of interest. Then, the researchers will evaluate the transgenic plants in the research greenhouse.

The project caps off with a field study, which sets it apart from most other projects. "It is almost always the case that performance under field conditions is different from performance in the greenhouse," says co-principal investigator Stephen DiFazio, a professor in plant genomics who will oversee the field trials at WVU.

The field exposes transgenic plants to other stresses, such as wind, frost, insects, and pathogens, not seen in the greenhouse environment. The field trials could reveal if altering the expression of a native gene disrupted another system of the plant, explains DiFazio.



Using laser capture microdissection, a scientist manually selects which leaf cells to harvest. Credit: Amir H. Ahkami | EMSL

Progress so far

The field study is still a couple years away, because the project is now only nearing the end of its first year. The project is challenging, says Ahkami, but he's optimistic.

"The data we've generated so far, particularly for proteomics, are very promising," he says. "The protein identifiers we found in each cell type provide proof of concept for a technique that could be broadly used for molecular phenotyping of poplar leaf and root tissues under stress at cell-level resolution."

Ahkami and the rest of the team at EMSL wouldn't be at this stage

without the ability to integrate multiple capabilities available at the user facility—some of which have never been combined before. Recently, EMSL announced a reorganization and realignment to new sciences areas, and its Biological Sciences Area aims, among other foci, to improve strategies for designing plants for biofuels productions, which is the SyPro Poplar project's objective.

Certain key tools and expertise at EMSL allow for the project's novel cell-type specific analysis. From the leaf and root samples, the researchers can target specific cells types for harvesting using laser capture microdissection or LCM. For example, a leaf has palisade and spongy mesophylls cells and vascular tissues, and a root has epidermis and cortex cells and stellar tissues. The high resolution of EMSL's LCM microscope lets the researchers manually select cells of a certain type. The system will cut the desired cells and then catapult them contact-free to a collection device.

By isolating the cell or tissue by type, "you're enriching your signal, isolating a much more specific population that you can then do your downstream applications and questions on," says Will Chrisler, PNNL's LCM expert. Using a bulk leaf or root sample could easily bury the signal.

Before proteomics analysis by mass spectrometry, the research team needs to prepare the cell-type specific samples collected by LCM. The newly developed technology called nanoPOTS (nanodroplet Processing in One pot for Trace Samples) plays a key role here.

"The bottleneck with proteomics was with sample processing," says analytical chemist Ryan Kelly, one of the nanoPOTS developers, who maintains a joint appointment with EMSL while now at Brigham Young University. It required many cells, because most of the sample was lost in moving from raw material to analysis-ready. Proteins cannot be

amplified as can DNA and RNA.

The sample processing technique nanoPOTS allows scientists to measure proteins in samples that are 100 to 1000 times smaller than they previously could. Credit: Andrea Starr | PNNL

But now nanoPOTS allows the team to measure proteins in samples that are 100 to 1000 times smaller than those used previously, explains Kelly.

The power of these tools is part of what allows a project of such magnitude to make such fast progress in only five years.

Popular poplar

Another factor that speeds up the work is the subject: the poplar tree.

One convenient characteristic is its ease in vegetative propagation, according to DiFazio. Stem cuttings placed in soil will easily root. Most trees take five years to flower, so waiting for seeds would take the length of the project, he explains. Instead, vegetative propagation allows the researchers to make hundreds or even thousands exact copies of the original within a couple of months. Plus, copies maintain the genetic improvements unlike seeds that could lose them from crossing with another parent plant.

That property, alongside its ease of managing in greenhouses and research settings, makes poplar a well-studied plant. Information on poplar is abundant, including its whole genome sequence. It also has an efficient transformation system, unlike most other trees.

Poplar is already widely distributed across the United States and Canada. But if the team is successful, says Ahkami, then growers can expand poplar to more areas and conditions where they currently do not thrive, making more biomass available.

DiFazio thinks of rural regions, like the coal communities in his home state of West Virginia, which are losing their traditional income sources. "Those communities would have the opportunity to have a big role in the energy economy, if we can develop crops that will grow on the marginal lands that are abundant in those parts of the country," he says.

One such site is the surface mines. "It completes the cycle," he says.

Soon on industrial lands, row after row of green energy could be peeking through.

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