Biotechnology Research is Developing New Tools for Tree Breeders

BY GLENN HOWE AND
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Forest biotechnology refers to the use of biological processes at the molecular or cellular level to make products or technologies useful to society. Here, we discuss the use of forest biotechnology to enhance tree breeding, focusing on molecular genetic markers, gene discovery, and genetic engineering.

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Molecular genetic markers have been used for at least two decades to enhance the production of genetically improved seed. Most molecular genetic markers are proteins (e.g., allozymes) or DNA segments that are used to track the inheritance of particular chromosomal locations. Markers can be used to simply determine how trees are related to one another, or to track specific genes. In the 1980s, allozyme markers began to be used to: (1) identify mislabeled trees in breeding populations and seed orchards; (2) determine the parents of open-pollinated seedlots; and (3) manage pollen contamination in seed orchards. Later, the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) developed simple sequence repeat (SSR) markers for Douglas-fir that are now widely used by breeders. SSRs largely replaced allozymes because they are easier to measure and are more accurate and precise for measuring genetic differences. Recently, the PNWTIRC developed hundreds of thousands of SNP (single nucleotide polymorphism) markers for Douglas-fir. Because SNPs are single-letter changes in the DNA code, they are easy to measure using automated technologies, and there is a vast number of them in the genome. SNPs have

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These eight-year-old poplars, genetically engineered for containment, are undergoing testing in a field trial at OSU. For scale, shown is six-foot-tall Thomas Howe.

also been developed for interior spruce and lodgepole pine by the University of British Columbia's AdapTree project.

SNP markers are particularly good for a breeding approach called genomic selection, where superior genotypes are identified by using thousands of SNP markers per tree. First, we construct a prediction equation using a training population of trees that has both SNP and trait data. Then, we apply the prediction equation to other populations (e.g., seedlings) where the traits have not been measured. Thus, instead of selecting trees based on measuring the traits directly, we can use SNPs. Eventually, it should become possible to use genomic selection to reduce field testing, select for mature tree traits (e.g., wood quality) at the seedling stage, and speed genetic improvement by reducing the length of the breeding cycle. While cost is still a key constraint, the cost of using DNA markers continues to decline because of advances in technology. In Douglas-

fir, genomic selection is being actively studied by the PNWTIRC and Northwest Tree Improvement Cooperative using funding from the USDA Northwest Advanced Renewables Alliance.

Sometimes it is important to know about specific genes. For example, single genes with large effects are occasionally found in native tree populations, and it is valuable to know exactly which genes these are. Once the genes have been discovered, genetic markers for these genes can be developed and used to guide tree breeding. This approach is being taken to develop SNP markers for the *Cr1* gene in sugar pine, a gene that confers resistance to white pine blister rust. Knowledge about single genes can also be used to alter the characteristics of trees using genetic engineering. This can be accomplished by inserting genes from other species, or by changing the expression of native genes in the target species.

Two approaches are commonly used to discover genes of interest. The

first approach is to look for correlations between gene expression and the trait of interest. The second approach is to look for genetic cosegregation using a genome-wide association study (GWAS). Using GWAS, we can test many thousands of genes to see which are inherited with particular traits from one generation to the next.

The first step in the pathway from gene to phenotype (the observable characteristics of a tree) is the synthesis of messenger RNA (mRNA). If we find a strong relationship between the amount of mRNA in a tree and a particular trait, the underlying gene is likely to be at least partly responsible for the resulting phenotype. Advanced technologies for mRNA sequencing now make it possible to conduct these studies on a genome-wide scale, using a method called transcriptomics. Tree samples are collected from particular tissues at specific times, mRNAs are isolated in the laboratory, the amounts of specific mRNAs are determined using sequencing machines, and the underlying genes are identified. We used this approach to discover genes associated with cycles of growth and dormancy in black cottonwood, and scientists at the USFS Pacific Northwest Research Station used a similar approach to study adaptation in Douglas-fir. The genes that we discovered are good candidates for influencing traits important to tree breeders, including genes associated with cold hardiness and drought hardiness.

Similar transcriptome studies by the Tree Biosafety and Genomics Research Cooperative (TBGRC) were used to identify genes involved in flowering, pollen formation, and seed production. The TBGRC used RNA sequencing to identify genes expressed in the developing flowers, pollen, and fruits of poplar and eucalypt trees. The transcriptome-identified genes are now being studied using genetic engineering.

A major TBGRC project is to develop reliable methods for preventing the spread of exotic and genetically engineered trees when required by regulations or for social license. Two highly effective genetic engineering methods are being studied, RNA interference (RNAi) and gene editing using CRISPR-Cas9 nucleases. Both methods are also widely used in medicine and agriculture, and are highly specific and efficient. RNAi poplars have been produced and found to be healthy and fully sterile in field trials. Gene edited trees have been produced in both poplar and eucalypts, and are currently being studied in the greenhouse.

A current effort in the forest biotechnology laboratory at OSU, funded by a major grant from the National Science Foundation (NSF), is to use GWAS to identify the genes that control capacity for genetic engineering in black cottonwoods. Many tree species and genotypes, even within poplars, are very difficult to genetically engineer, and the reasons are largely unknown. By identifying the genes involved, researchers will gain new insights into the nature of the constraints and produce new "gene reagents" that could be used to overcome barriers in poplar or other species.

TBGRC and forest biotechnology staff are also active in education about GMOs in general, and genetic engineering of trees in particular. As part of the new NSF grant, we are developing new methods for teaching high school students and teachers about genetic engineering methods and impacts. This work, broader outreach efforts, and stewardship technology such as genetic containment methods, are essential to develop the social license to use genetic engineering in forestry. ◆

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SAF National Convention Coming to Portland in 2018

Mark your calendar for October 3-7, 2018, when the largest gathering of foresters in the country will converge on Portland for the annual SAF national convention.

While the 2017 national convention in Albuquerque is right around the corner, Northwest members should be aware of this national convention planning effort as many may be interested in volunteering and attending while it's in our backyard.

Tammy Cushing is the general chair for convention. Volunteer opportunities will be available before and during the convention. From organizing field trips to staffing the raffle, there will be chances for members to contribute. If you are interested in volunteering, send an email to Tammy at tamara.cushing@oregonstate.edu. Volunteer service is a great way to meet people and show Northwest hospitality.

Tammy Cushing

Convention typically draws over 1,600 professionals and students, and we are expecting a larger crowd in Portland. Foresters and natural resource professionals gather at this meeting to hear the latest research results, network, learn about local forest management and practices, get outside and attend field trips, present posters, chat with vendors showing new technologies and services, gain continuing education credits, and collaborate for the future.

Be sure to take advantage of the convention being in the Northwest and let's show forestry professionals from around the nation our amazing forest resources. Additional information will be shared as we get closer to the event. ◆