Trees of the Future - Genetic Engineering as a Tool for Improving Feedstock

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Presentation overview

- Rationale
- Poplar improvement: what are the options?
- Regulatory and social concerns
- Data: what do we know so far?
- Future prospects
Rationale

- Genetic engineering (GE): a valuable tool for economics and sustainability
  - Improved feedstock yield (biomass)
  - Feedstock quality modification
    - Increase in product formation (fermentability)
    - Input reduction (pesticides, irrigation)
    - Valuable co-products (chemicals, bioplastics, enzymes)
- Social, marketplace, and regulatory limits on use of GE are severe (e.g., FSC system)
- Gene flow to certified and wild stands a major issue
We know lots of information about poplar genetics

- First ever sequenced tree genome was *Populus trichocarpa*
- Lots of naturally existing genetic diversity

The Genome of Black Cottonwood, *Populus trichocarpa* (Torr. & Gray)

Plantations face many stresses

- Insect pests
- Diseases
- Drought
- Weeds
- More animal pests
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Poplar improvement: how to obtain a better tree

- Option 1: traditional breeding
- Option 2: genetic engineering
Genetic engineering can quickly add specific traits to trees

New DNA can encode a new trait of interest (insect resistance) or alter a native gene

GE can add traits faster and more specifically than traditional breeding
Genetic engineering had led to biofuel feedstock benefits in poplar.
Genetic engineering can be used to obtain high value traits in poplar.

- Insect resistant
- Not insect resistant
Genetic engineering can make poplar trees with specialty chemicals

- Trees produce 2-phenylethanol, a chemical with multiple uses and a high-value
- This chemical is normally found in roses and other flowers

Transgenic Hybrid Poplar for Sustainable and Scalable Production of the Commodity/Specialty Chemical, 2-Phenylethanol

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Abstract

Fast growing hybrid poplar offers the means for sustainable production of specialty and commodity chemicals, in addition to rapid biomass production for lignocellulosic deconstruction. Herein we describe transformation of fast-growing transgenic hybrid poplar lines to produce 2-phenylethanol, this being an important fragrance, flavor, aroma, and commodity chemical. It is also readily converted into styrene or ethyl benzene, the latter being an important commodity aviation fuel component. Introducing this biochemical pathway into hybrid poplars marks the beginnings of developing a platform for a sustainable chemical delivery system to afford this and other valuable specialty/commodity chemicals at the scale and cost needed. These modified plant lines mainly sequester 2-phenylethanol via carbohydrate and other covalently linked derivatives, thereby providing an additional advantage of effective storage until needed. The future potential of this technology is discussed. MALDI metabolite tissue imaging also established localization of these metabolites in the leaf vasculature.
Genetic engineering can create trees with valuable co-products

- Addition of 3 genes from bacterial to poplar can lead to the formation of biodegradable plastic in trees
- Current methods have a tradeoff between polymer production and plant growth

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**Trade-offs between biomass growth and inducible biosynthesis of polyhydroxybutyrate in transgenic poplar**

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There are public and social concerns over the use of GE trees.

“The potential loss of biodiversity due to a risk of gene flow between transgenic and wild trees was seen as the safety issue of most concern when considering the commercial release of transgenic forest trees.”
Gene flow: the movement of pollen and/or seeds into new populations
Lesson from crops: weed control measures used with resistant crops led to selection for resistant weeds.

We want to avoid similar problems with any potential future use of GE trees.
Many poplar hybrids are crosses between exotic species.

- *Populus nigra*
- *Populus deltoides*
- *Populus maximowiczii*
- *Populus trichocarpa*
Forest stewardship council: concern over potential ecological impacts of exotic tree species

“The use of exotic species shall be carefully controlled and actively monitored to avoid adverse ecological impacts”

Traditional breeding has not yet been able to achieve a solution to this issue. GE methods can prevent spread by pollen and/or seeds.
Genetic containment as a possible solution to prevent spread of GE and exotic species

Goal: create non-flowering trees

Pollen and seeds can spread for miles on the wind

Long-distance impacts across many boundaries
We are targeting fundamental regulators of flowering by RNA interference (RNAi)

Scientists have identified key genes for the development of flowers
Gene suppression with RNAi (RNA interference) has already produced diverse traits.
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Strauss lab RNAi trees are undergoing field testing

5 FT 2 inches
RNA interference again the key floral regulator LFY led to female sterile trees

Containment of transgenic trees by suppression of **LEAFY**

To the Editor:

Field studies and commercial use of genetically engineered (GE) trees have been limited, in large part owing to concerns over transgene flow into wild or feral tree populations. Unlike other crops, trees are long-lived, weakly domesticated and their propagules can spread over several kilometers. Although male sterility has been engineered in pine, poplar, and eucalyptus trees grown under field conditions by expression of the barnase RNase gene in anther petal cells, barnase can reduce rates of genetic transformation and vegetative growth. Furthermore, barnase expression may not be fully stable. Bisexual sterility would allay concerns over seed dispersal, could be used to control invasive exotic trees, and might increase wood production. We report the use of RNA interference (RNAi) to suppress expression of the single-copy *LEAFY* (*LFY*) gene to produce sterility in poplar. RNAi has been used to reduce gene expression in many plant species, and the reduction in gene expression that RNAi confers is highly stable in trees under field conditions. *LFY* is required for the early stages of male and female floral organ formation in plants, and encodes a transcription factor that promotes floral meristem identity. In *Arabidopsis thaliana*, loss of *LFY* function results in the formation of vegetative structures instead of floral meristems, whereas reduction of *LFY* expression decreases floral abundance and results in partial conversion of floral organs to leaf-like structures. We selected *LFY* for suppression in poplar.

http://hardwoodbiofuels.org/news-fall2016-poplar-reproduction/

Klocko et al. Nature Biotechnology 2016
RNAi-LFY trees appear to be female sterile

Flowers fail to enlarge and were seedless
Female sterile RNAi-LFY trees have normal vegetative performance
Sterile trees can still be propagated by vegetative cuttings

Healthy new trees were started by rooting branches of sterile trees
We are also trying the latest and greatest method for targeting floral genes.

Science magazine names CRISPR ‘Breakthrough of the Year’

By Robert Sanders | DECEMBER 18, 2015

In its year-end issue, the journal Science chose the CRISPR genome-editing technology invented at UC Berkeley 2015’s Breakthrough of the Year.

A runner-up in 2012 and 2013, the technology now revolutionizing genetic research and gene therapy “broke away from the pack, revealing its true power in a series of spectacular achievements,” wrote Science correspondent John Travis in the Dec. 18 issue. These included “the creation of a long-sought ‘gene drive’ that could be used to eradicate malaria

Growing two-dimensional bone
Managing biological risk
Algal toxins alters spatial memory
The CRISPR method can be used to create organisms not regulated as GE by the USDA.

These mushrooms have a precise change to a single mushroom gene.

No extra DNA/genes are present.

Similar applications in trees might be acceptable by the FSC or other regulators.
Upcoming Strauss lab research:

- Scaled-up field test of RNAi-LFY trees
- Greenhouse and field tests of trees with floral genes targeted by CRISPR
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Future use of genetic containment in poplar trees could benefit biofuel plantations

- All AHB field sites are non-GE hybrids of poplar species
- If exotic species become a concern or if GE varieties are tested, containment could be used to
  - Prevent flowering if fields have early flowering or aren’t harvested as planned
  - Prevent movement of pollen and seeds to wild populations, feral trees, or other plantations
Future prospects

- GE traits can provide considerable value and benefits to poplar plantations
- Obstacles prevent commercial use – gene flow is a major concern
- Our research established that sterility can be a solution
- New developments in gene editing technology (CRISPR) offer possibilities for tree containment
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