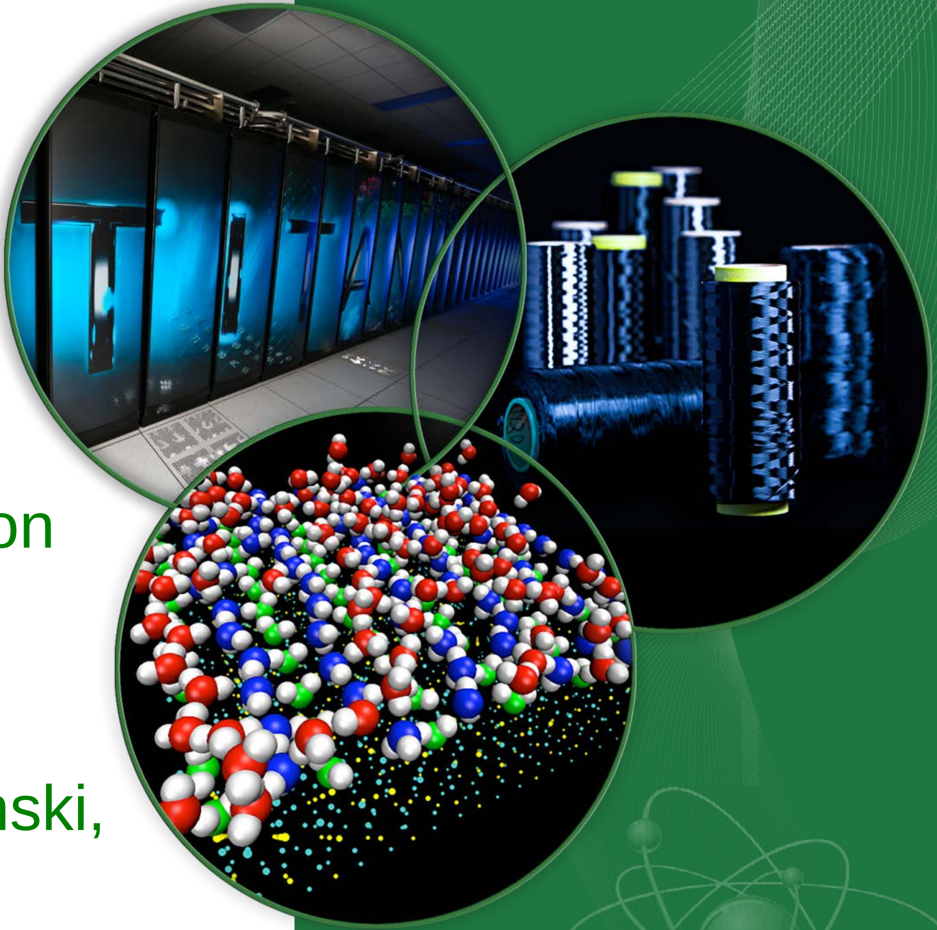
A Gene for Regulating Phenylpropanoid, Tryrosine and Tryptophan Pathways



Principal Investigator: Wellington Muchero

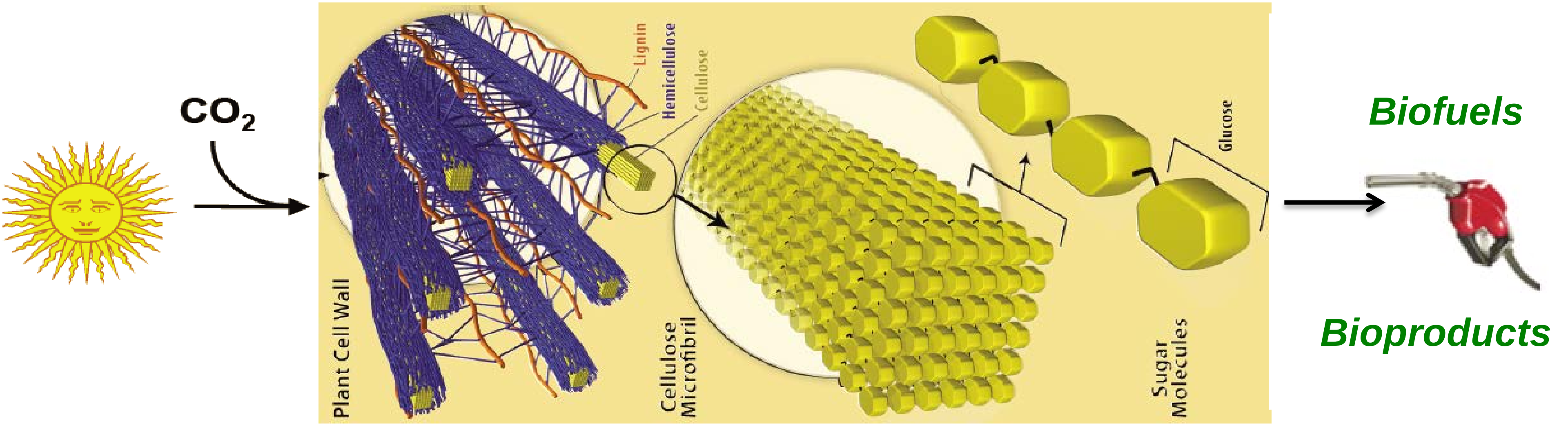
Lead Division: Biosciences

Co-Investigators: T. J Tschaplinski,

J-G Chen, G.A Tuskan

Challenge: Prohibitive Pretreatment and Enzyme Costs

LIGNIN and Hemicellulose: ***The > $750 Million obstacle***



Costly pretreatment and enzymatic digestion

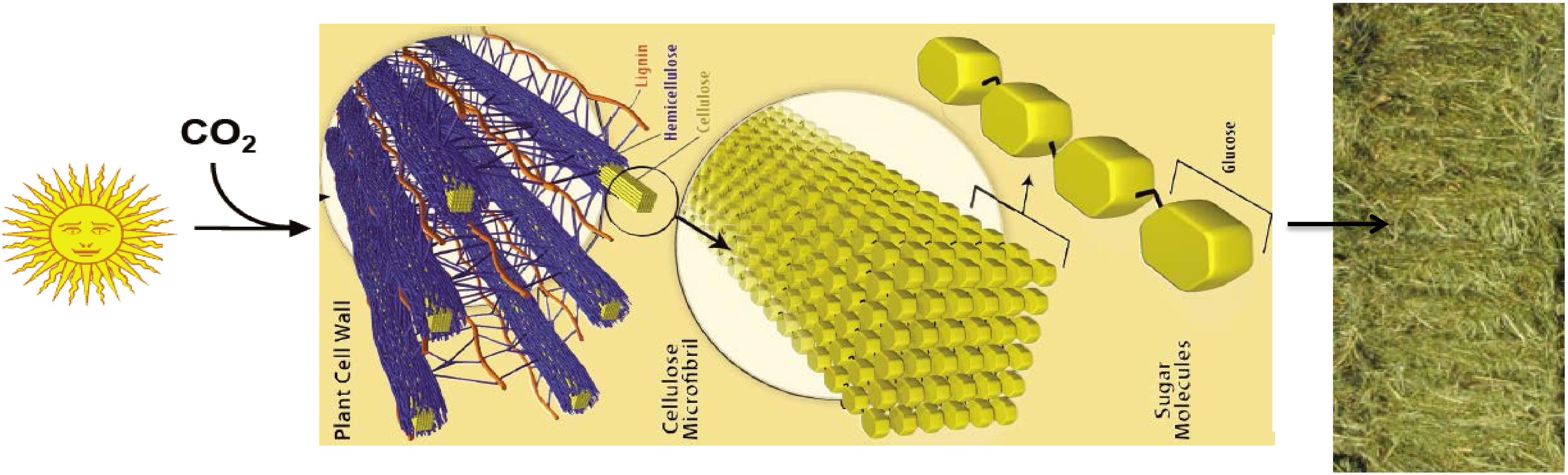
U.S. DEPARTMENT OF ENERGY

BESC BioEnergy Science Center

OAK RIDGE National Laboratory

Challenge: Prohibitive Pretreatment and Enzyme Costs

<u>LIGNIN and Hemicellulose:</u> ***The > $750 Million obstacle***



Incomplete digestion and nutrient loss

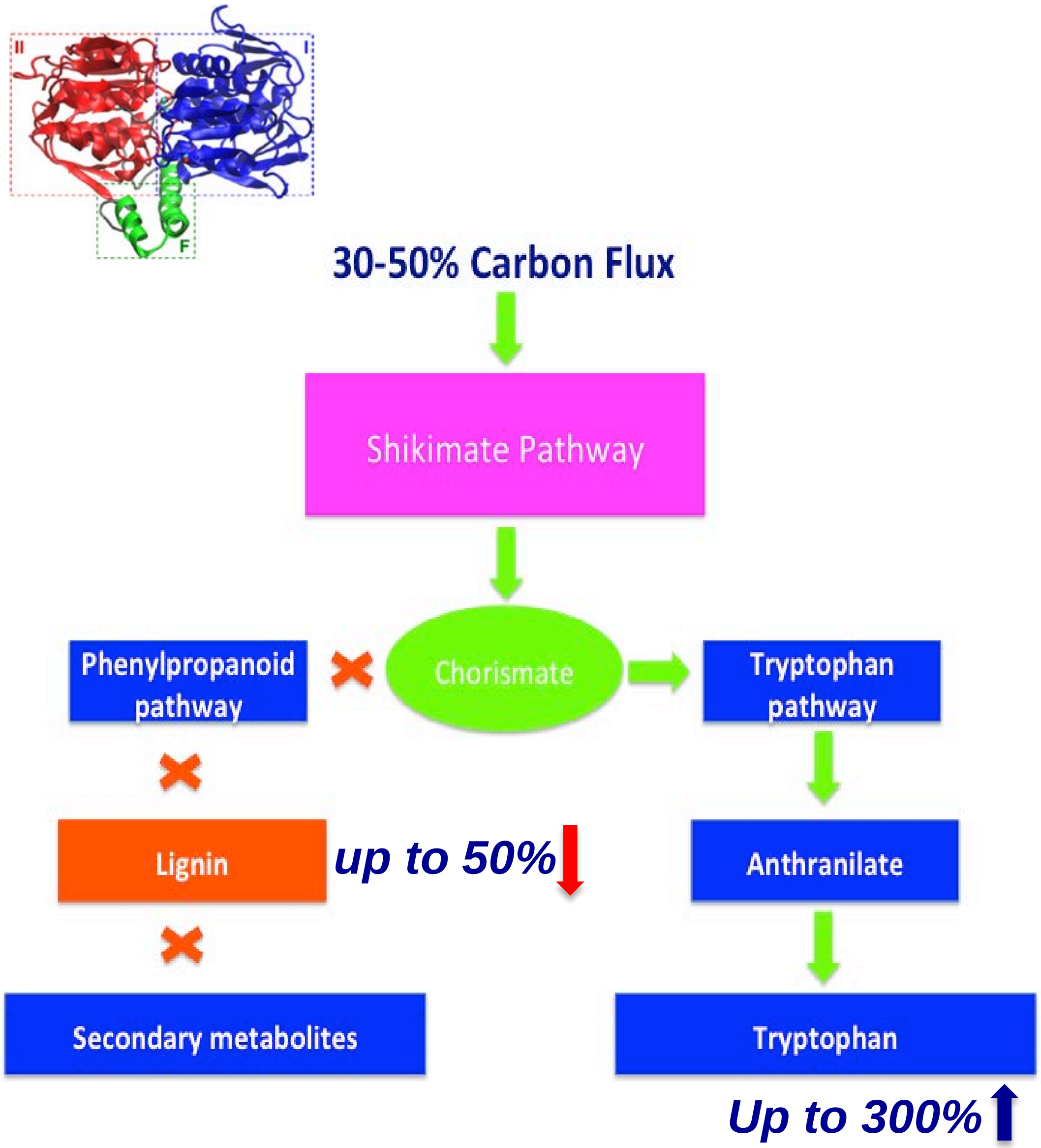
Forage quality

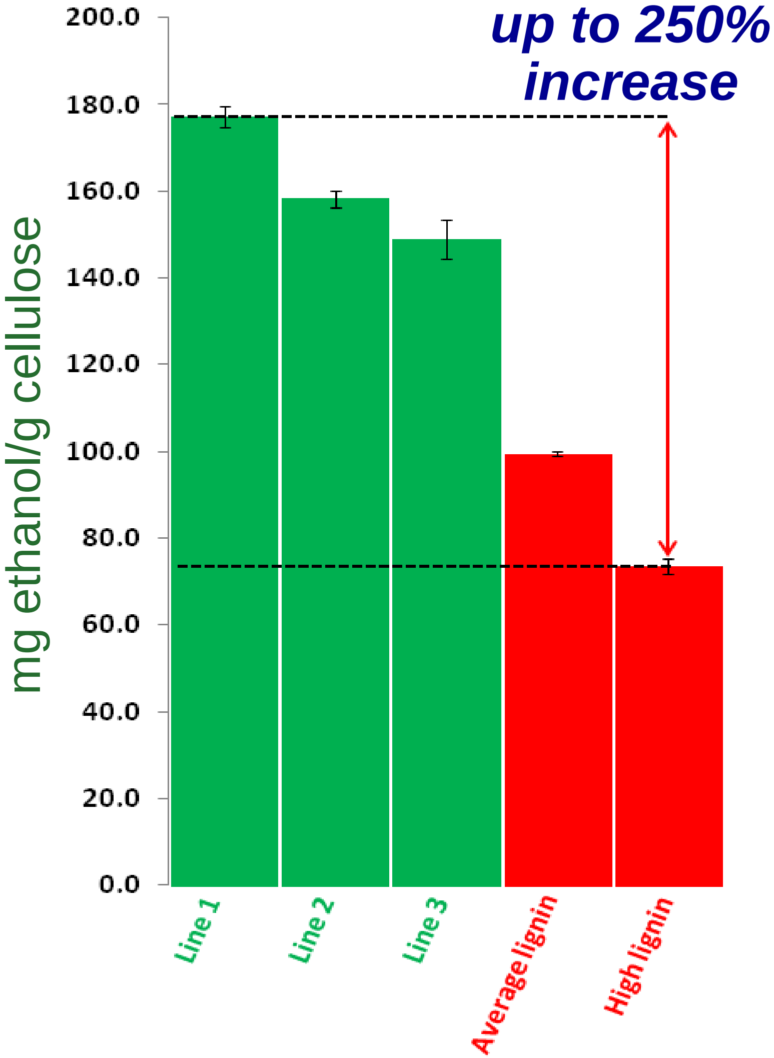
U.S. DEPARTMENT OF ENERGY

BESC BioEnergy Science Center

Oak Ridge National Laboratory

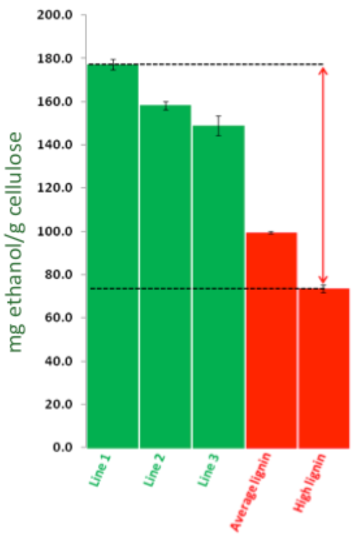
Novel Protein Reduces Lignin and Increases Ethanol Yield



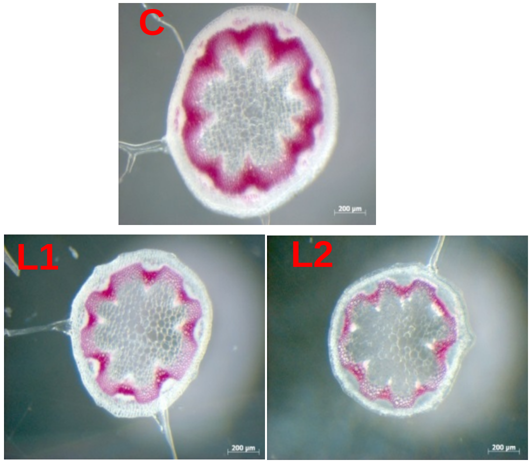


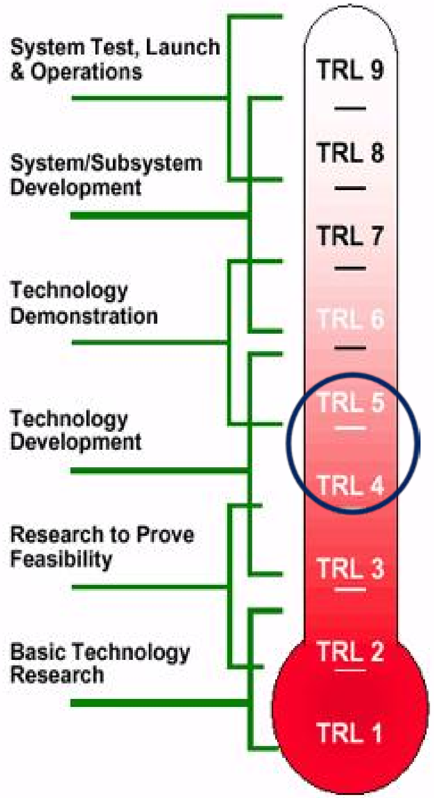
Technology Development Stage: Proven in *Populus* and *Arabidopsis*

5-yr –old unpretreated Populus tree



Arabidopsis





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Technology Leadership

Why is this technology unique?

- The target protein isoform has *not been characterized in any biological system*.
- The first demonstration of *a carbon-gating function* between *3 critical pathways* in any plant systems using a single gene-target.
- *No biomass yield penalty* under production conditions

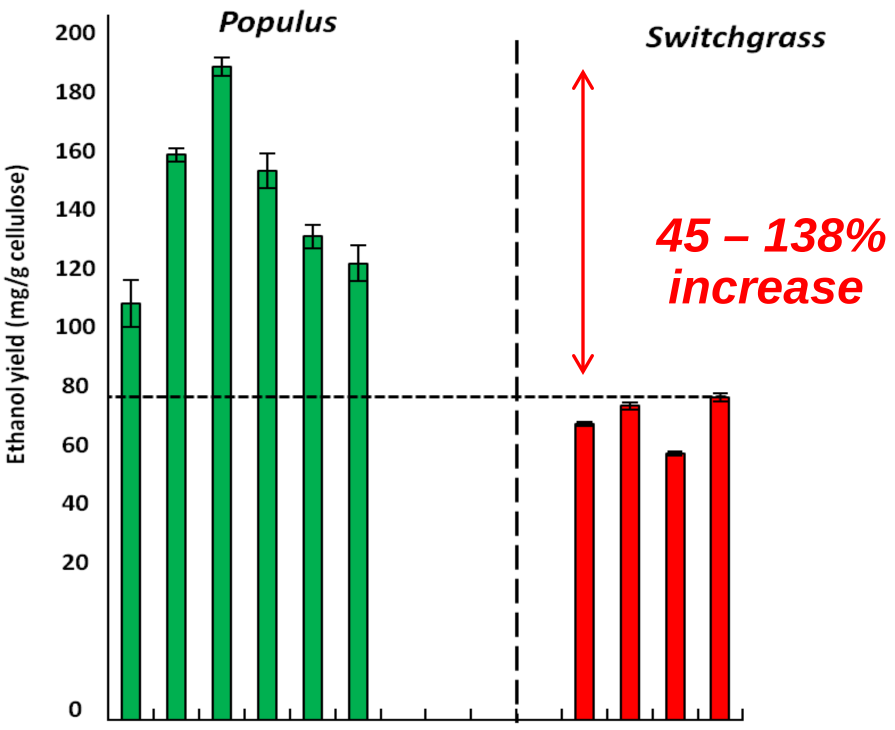
What are the performance indicators that show that this new technology works better than the existing technology and methods?

- Enhanced *ethanol yield* (*up to 250% increase*)
- Reduced *energy and capital costs* by reducing pretreatment severity (*up to 30% reduction in materials costs*).
- Reduced *enzyme costs* (*more than 300% reduction*).

Comparable technology

…"*increases the ethanol yield by up to 38%* using conventional biomass fermentation processes. The down-regulated lines *require less severe pretreatment and 300–400% lower cellulase* dosages for equivalent product yields…." Fu et al. (2011) *PNAS.*

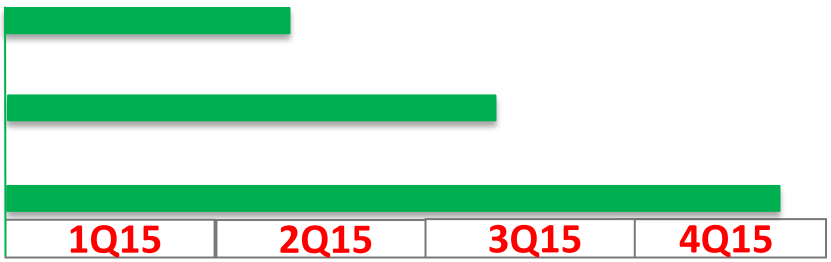
vs



Research and Development Plan

Describe the proposed technical research and development plan, including approach, milestones and deliverables.

- Validation *in Arabidopsis (model plant)*
- Validation in Rice (*model for monocots*)
- Validation in Medicago (*model for forage*)

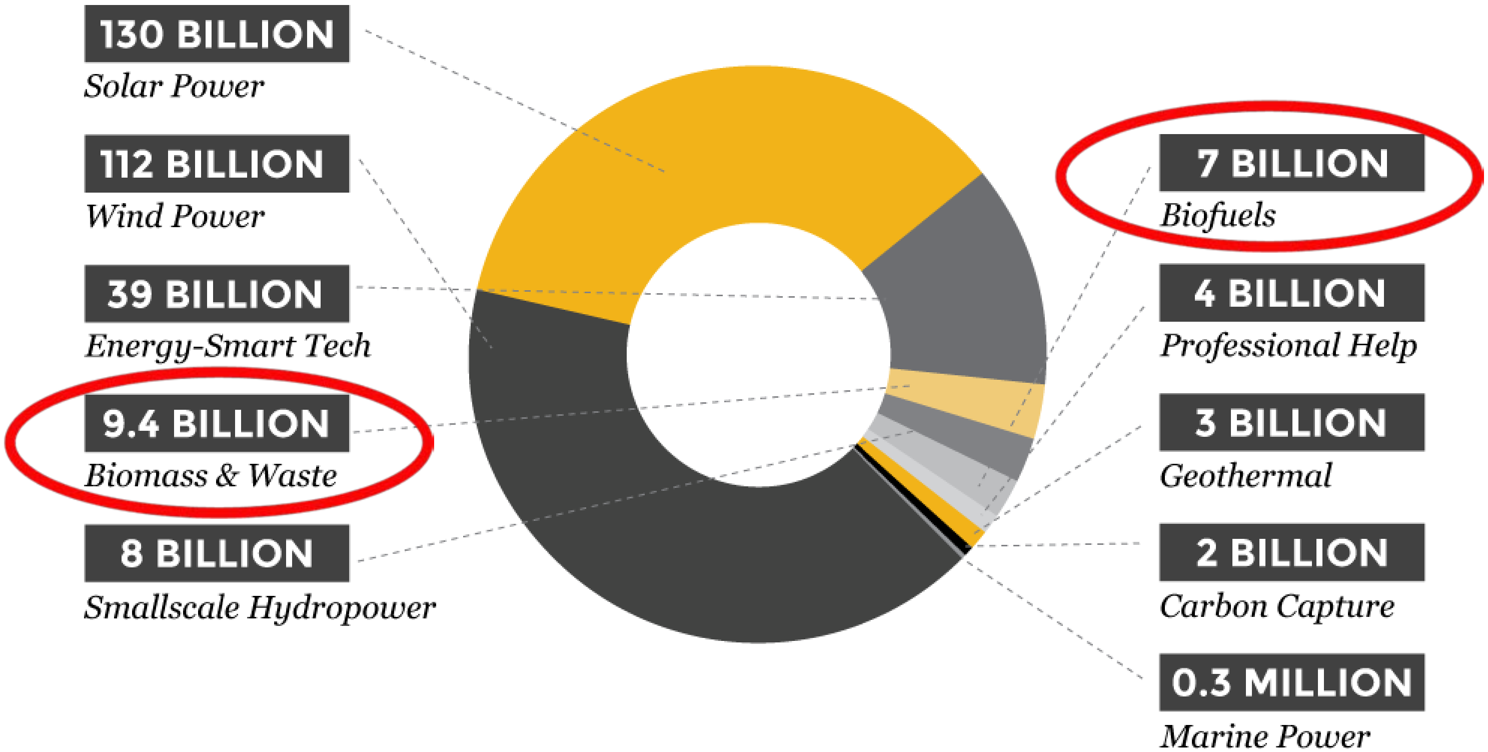


What are the key challengers that must be overcome for the technology to have the anticipated impact?

- Deliverable 1: Demonstrate reduced expression of PAL and TAL genes and enhanced expression of the AS gene in target crops.

- Deliverable 2: Demonstrate viability of progeny and inheritance of gene regulatory activities.

Commercialization Plan: Clean Energy Opportunities



314 Billion New Energy Dollars Were Invested Worldwide in 2013

CATALYZING THE CLEAN TRILLION

SOURCE: BLOOMBERG ENERGY FINANCE, April 7, 2014

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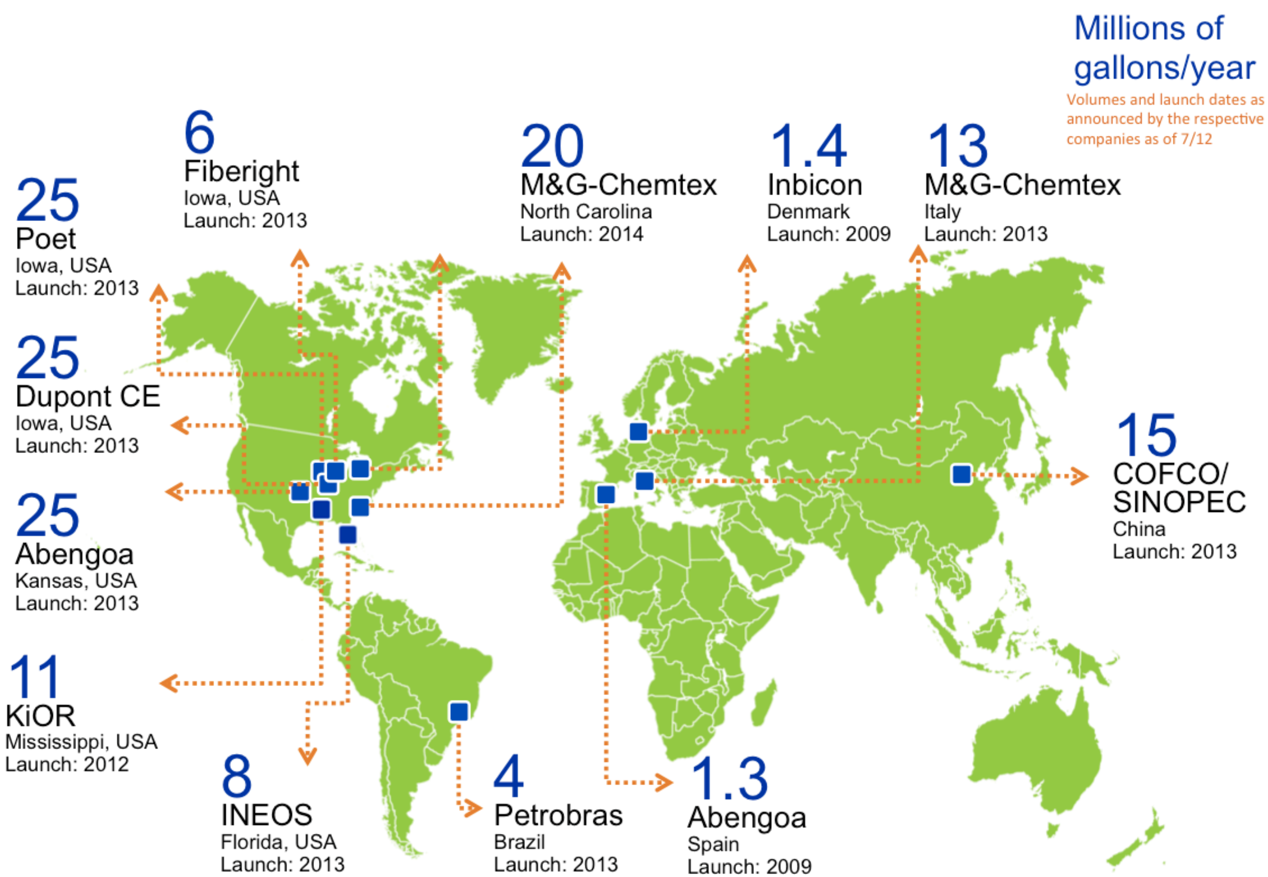
Applications – Target Customers – Current Practice

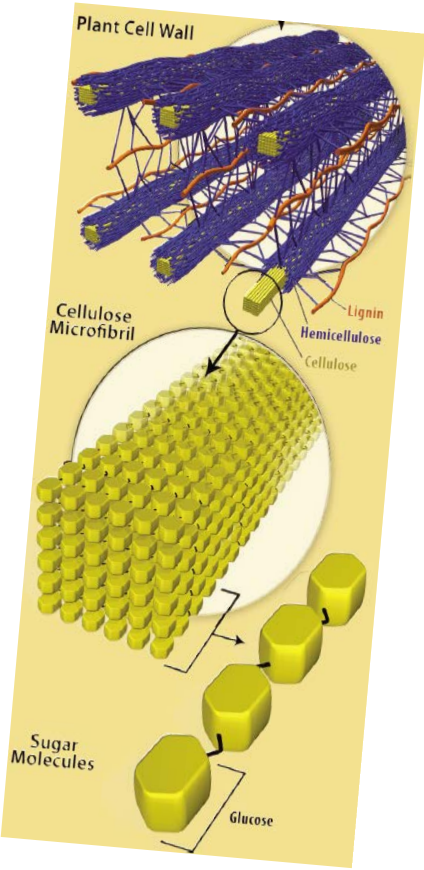
	Application Description	Target Customers	Current Practice
Application 1	BioFuels / Pulping	List Below	Severe pretreatment



Total projected capacity ~155 M gal/y

Other Applications

	Application Description	Target Customers
Application 2	**Forage digestibility**	**Agricultural Biotechnology Sector**



Source: Westernfarmpress

Intellectual Property Available for Licensing

US Provisional Patent Application 62/008,434
Filed June 5, 2014

"An EPSP Enzyme Which Regulates Phenylpropanoid, Tyrosine and Tryptophan Pathways,"

Wellington Muchero, Jay Chen, Lee E. Gunter, Sara Jawdy, Gerald A. Tuskan, Anthony Christian Bryan, Stephen Difazio, and Hao-Bo Guo.

UT-Battelle Invention No. 201403346

A Gene for Reduction of Lignin Content in Plants

UT-B ID 201403346

Advantages

- Enhanced plant performance without undesirable characteristics
- Ability to target multiple metabolic pathways with a single gene
- Enhanced nutritional quality

Potential Applications

- Bioenergy and bioproducts
- Cellulosic ethanol production
- Forage improvement
- Disease and insect resistance
- Tissue-specific gene expression
- Multiple pathway regulation

Patent

Wellington Muchero, Jay Chen, Lee E. Gunter, Sara Jawdy, Gerald A. Tuskan, Anthony Christian Bryan, Stephen Difazio, and Hao-Bo Guo. *An EPSP Enzyme Which Regulates Phenylpropanoid, Tyrosine and Tryptophan Pathways*, Provisional US Patent Application 62/008,434, filed June 5, 2014.

Inventor Point of Contact

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Licensing Contact

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Technology Summary

One of the biggest challenges in making biofuels viable is finding the most economical way to extract the maximum amount of fuel from plant biomass. The volume of fuel that can be produced from a plant depends primarily on how easily the plant's cell walls can be broken down—or degraded. However, because cell walls are recalcitrant (i.e., resistant to degradation), a main factor in enabling economic conversion of plant biomass to biofuels is finding genetic solutions to recalcitrance. The same challenge is faced in animal feed crops where cell wall recalcitrance reduces the nutritional quality of forage crops. Researchers at ORNL used an innovative process called association genetics to quickly identify a gene that affects cell wall recalcitrance. This process allowed scientists to complete their task within a matter of weeks, compared with previous, more cumbersome methods that can take decades.

Researchers studied several varieties of Poplar trees grown throughout the United States to understand the genetics behind why there is such a wide variation in the biomass produced by the same type of trees grown in the same environment. To do this, they evaluated how the genetic makeup, or genotype, of the trees interacts with the natural environment to produce certain observable traits, or phenotypes. After data gathering and analysis, a gene was identified as one of those responsible for controlling recalcitrance-related phenotypes in Poplar trees.

The newly identified gene can be manipulated in Poplar trees, Eucalyptus, alfalfa, rice, and other plants to enhance plant performance without producing undesirable phenotypes. Such manipulation alters cell wall chemistry, resulting in reduced resistance to sugar release from cell wall components. In turn, this can lead to an advantageous conversion rate of plant biomass to cellulosic ethanol. The same benefits accrue in forage crops for animal feed with enhanced nutritional quality resulting from reduced lignin content. In addition, altering the expression of the identified gene can provide other benefits, including enhanced resistance to insects and microbial pathogens such as fungi and bacteria.

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MANAGED BY UT-BATTELLE FOR THE US DEPARTMENT OF ENERGY

To view this and other ORNL inventions, visit ORNL Partnerships at http://www.ornl.gov/connect-with-ornl/for-industry/partnerships/technology-licensing/available-technologies

03.2015

Competitive Differentiation

Technology Feature	ORNL Technology	Chemical pretreatment	Mechanical Pretreatment
Enzyme Reduction	✓	✓	✓
Low Energy Input	✓	✗	✗
Non-Corrosive	✓	✗	✓
Reduce Lignin Waste	✓	✗	✗
Reduce Capital Costs	✓	✗	✗

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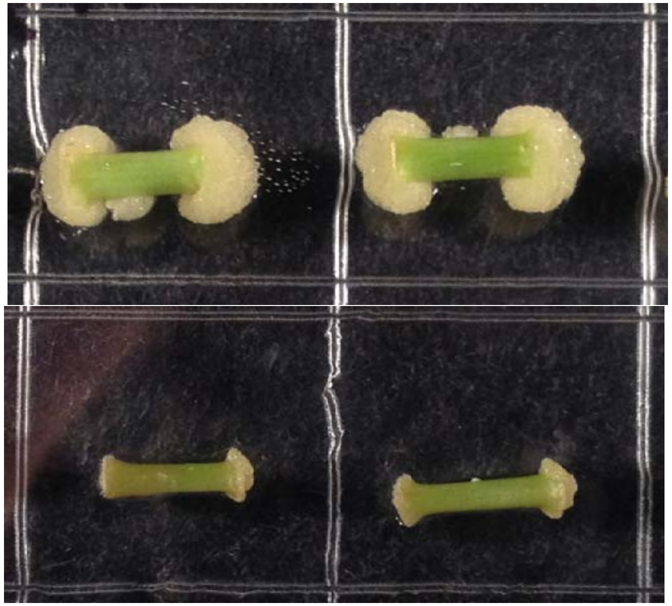
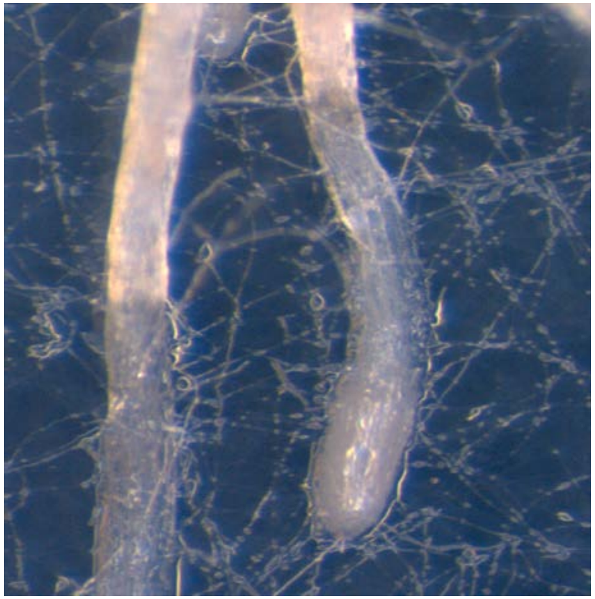
Pathogen resistance

Tumor regulation



Symbiotic interactions



Biomass yield

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