

Plant Feedstock Genomics for Bioenergy Joint Awards

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U.S. Department of Agriculture



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Integrating the U.S. Department of Energy's (DOE) capabilities in genomic sequencing and analysis for production of biofuels with the U.S. Department of Agriculture's (USDA) long experience in crop improvement, DOE and USDA are working together to fund projects that accelerate plant breeding programs and improve bioenergy feedstocks. Investigators in these projects are characterizing the genes, proteins, and molecular interactions that influence lignocellulosic biomass production.

Significant advances in breeding, molecular genetics, and genomic technologies provide an opportunity to build upon the existing knowledgebase of plant biology to be able to confidently predict and manipulate their biological function for bioenergy resources. Specific areas of interest include:

- Elucidation of the regulation of gene networks, proteins, and metabolites
 - To improve plant feedstock productivity, sustainability, water use efficiency, and nutrient utilization.
 - To advance understanding of carbon partitioning and nutrient cycling in plant feedstocks.
- Comparative approaches to enhance fundamental knowledge of the structure, function, and organization of plant genomes leading to innovative strategies for feedstock characterization, breeding, or manipulation.

Since 2006, dozens of projects have been funded by the DOE-USDA Plant Feedstock Genomics program, which is jointly supported by the Office of Biological and Environmental Research within DOE's Office of Science and the USDA National Institute of Food and Agriculture (formerly the USDA Cooperative State Research, Education, and Extension Service). To advance the fundamental understanding of lignocellulosic biomass accumulation and other crop traits relevant to biofuel production, several plants have been investigated with support from this program. These include poplar (*Populus trichocarpa*), switchgrass, the model grass *Brachypodium distachyon*, *Miscanthus*, foxtail millet, rice, sorghum, alfalfa, sunflower, and maize.



Switchgrass image courtesy of Lawrence Berkeley National Laboratory.

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More details including Co-PIs and funding amounts are on the web at <http://GenomicScience.energy.gov/research/DOEUSDA/>

2010 Awards

Nine Projects Selected for Awards Totaling \$9 Million

Genome-Wide Analysis of miRNA Targets in *Brachypodium* and Biomass Energy Crops

- Pamela J. Green, University of Delaware, Newark

Goal: Identify the targets of MicroRNAs (miRNAs) in different organs and under adverse environmental conditions in the model grass *Brachypodium* and in the energy crops switchgrass, *Miscanthus*, and sorghum. miRNAs are important regulatory molecules that repress selected “target” genes to enable normal development, stress responses, and other processes. This project should enhance understanding of regulatory networks and may suggest new strategies for improving biomass energy crops.

Organ and Tissue-Specific Sucrose Transporters: Important Hubs in Gene and Metabolite Networks Regulating Carbon Use in Wood-Forming Tissues of *Populus*

- Scott A. Harding, University of Georgia, Athens

Goal: Investigate how sucrose transporter proteins (SUTs) function to facilitate the distribution of sucrose for transient storage and biosynthetic use among different pathways in the developing wood matrix. Wood for lignocellulosic feedstocks is synthesized from sucrose that is exported from leaves and then processed in the wood-forming organs. SUTs mediate the export and efficient movement of sucrose from source leaves to sink organs in all plant species.

The Role of Small RNA in Biomass Deposition and Perenniality in *Andropogoneae* Feedstocks

- Matthew E. Hudson, Energy Bioscience Institute, University of Illinois at Urbana-Champaign

Goal: Investigate the role of small RNA molecules in biomass production and their importance in the regulation of cellulose and lignin biosynthesis. The tissues and organs of next-generation biofuel crops that provide biomass for energy production are primarily composed of lignin and cellulose. This research will focus on *Miscanthus* species as well as other biomass crops including switchgrass and prairie cordgrass.

Development of a Low Input and Sustainable Switchgrass Feedstock Production System Utilizing Beneficial Bacterial Endophytes

- Chuansheng Mei, The Institute for Advanced Learning and Research

Goal: Understand the molecular and physiological mechanisms by which interaction with bacterial endophytes promotes growth in the promising bioenergy crop switchgrass. The use of naturally occurring beneficial bacterial endophytes with

switchgrass represents a practical and feasible way to develop a low-input and sustainable feedstock production system.

Functional Analysis of Regulatory Networks Linking Shoot Maturation, Stem Carbon Partitioning, and Nutrient Utilization in Sorghum

- Stephen Moose, University of Illinois at Urbana-Champaign

Goals: Determine if changes in the Glossy15 gene system of sorghum might contribute to current physiological differences among grain, sweet and biomass sorghums, and whether this gene can be used to convert superior sorghum grain hybrids to cultivars enhanced for bioenergy production.

Genomics of Energy Sorghum Biomass Accumulation

- John Mullet, Texas A&M University, College Station

Goal: Identify the genetic and biochemical basis for increasing yield and improving the composition of high-biomass cellulosic energy sorghum. Select genotypes will be analyzed for stem biomass yield, structure, and composition. The resources developed will enable analysis of the genes that modulate these traits and facilitate improvement of energy sorghum and other bioenergy grasses.

Identification and Genetic Characterization of Maize Cell-Wall Variation for Improved Biorefinery Feedstock Characteristics

- Markus Pauly, University of California, Berkeley

Goal: Identify and characterize maize lines with enhanced biorefinery feedstock characteristics, particularly those containing higher yields of fermentable sugars. Stover, the material from the corn plant that remains after removal of the grain, consists primarily of cellulose, hemicellulose, and lignin. Because corn stover is generated by U.S. agriculture in significant amounts, this lignocellulosic residue is desirable to use as a biofuel source.

Systems View of Root Hair Response to Abiotic Stress

- Gary Stacey, University of Missouri, Columbia

Goal: Gain insight into the impacts of variations in temperature and water availability on nutrient uptake by root cells. Root hair cells function to increase root surface area and to mediate water and nutrient uptake. The data obtained in this project should provide a better understanding of the impacts of climate change (heat and water limitation) on plant root physiology.

Insertional Mutagenesis of *Brachypodium distachyon*

- John P. Vogel, USDA Agricultural Research Service
Western Regional Research Center, Albany, Calif.

Goals: Generate 30,000 additional insertional mutants in the model grass *Brachypodium distachyon* and sequence DNA flanking the insertion sites. Insertional mutants are a powerful research tool that allow researchers to rapidly determine the function of specific genes. Mutants from outside collaborators will be integrated into this collection and made available through a public database.

2009 Awards

Seven Projects Selected for Awards Totaling \$6.3 Million

Improving Alfalfa as a Biofuel Feedstock

- E. Charles Brummer, University of Georgia, Athens

Goal: Develop in the long term biofuel-ready alfalfa cultivars that have improved yield and quality. Biofuel crops must maximize the production of energy, which requires a high yield of biomass with optimum fuel quality. In this project, molecular markers that are associated with optimal biofuel characteristics will be identified in alfalfa and directly integrated into traditional field-oriented alfalfa breeding programs.

A Systems Biology Approach to Elucidate Regulation of Root Development in *Populus*

- Victor Busov, Michigan Technological University

Goal: Identify key regulators of root architecture in relation to nitrogen and water use in the bioenergy crop *Populus* using an integrated systems biology approach. This research will generate resources and innovations that can enable robust biomass productivity under marginal conditions for sustainable lignocellulosic biomass production.



Poplar leaf image courtesy of the DOE Joint Genome Institute.

Characterization of Nitrogen Use Efficiency in Sweet Sorghum

- Ismail Dweikat, University of Nebraska, Lincoln

Goal: Enhance the ability of sweet sorghum to utilize nitrogen, increasing its potential as a leading and cost-effective bioenergy crop. This project will identify novel nitrogen use efficiency alleles in wild sorghum germplasm that can be used to improve sweet sorghum.



Sorghum image courtesy of Lawrence Berkeley National Laboratory.

Mechanism of Carbon Partitioning Regulation by *cpg13* in the Bioenergy Woody Crop Poplar

- Matias Kirst, University of Florida

Goal: Characterize genes that regulate the balance of carbon going to cellulose or lignin, leading to the development of plant materials that are more suitable for biofuel production.

Accelerating the Domestication of *Miscanthus* for Biofuel Production

- Andrew H. Paterson, University of Georgia, Athens

Goal: Provide genomic tools and resources for a promising cellulosic biofuel crop, *Miscanthus*, that will (a) foster innovative strategies for its improvement and (b) develop comparative and bioinformatic approaches to enhance fundamental knowledge of *Miscanthus* genome structure, function, and organization.

The Hunt for Green Every April: Factors Affecting Fitness in Switchgrass

- Gautam Sarath, USDA Agricultural Research Service (ARS), Lincoln

Goal: Investigate winter survival in switchgrass populations and individual plants specifically selected for greater yields and with known differences in winter survival. Molecular events occurring in the crowns and rhizomes will be studied over two growing seasons and winters. The project will make a significant and lasting contribution to the future improvement of switchgrass as a bioenergy crop, also directly benefiting researchers working on developing other perennial grasses into biomass energy crops.

Phenomic Analysis of Natural and Induced Variation in *Brachypodium distachyon*

- John P. Vogel, USDA Agricultural Research Service Western Regional Research Center, Albany, Calif.

Goal: Conduct high-throughput phenotypic analysis (phenomics) of homozygous T-DNA mutants and natural accessions of the model grass *Brachypodium distachyon* to accelerate the understanding of the basic biology underlying traits that control the utility of grasses as energy crops.

2008 Awards

Ten Projects Selected for Awards Totaling \$10.8 Million

Development of Genomic and Genetic Tools for Foxtail Millet, and Use of These Tools in the Improvement of Biomass Production for Bioenergy Crops

- Jeff Bennetzen, University of Georgia

Goal: Generate a variety of genomic and genetic tools for foxtail millet, including SNPs, BAC libraries, optimized foxtail millet transformation technology, and a high-density QTL and genetic map of foxtail millet for significant biomass traits. These resources will complement the DOE Joint Genome Institute whole-genome sequencing of foxtail millet, enhancing its value as a functional genomic model for second-generation bioenergy crops such as switchgrass.

Identifying Genes Controlling Ferulate Cross-Link Formation in Grass Cell Walls

- Marcia Maria de Oliveira Buanafina, Pennsylvania State University

Goal: Investigate the regulation of ferulic acid cross-linking in the cell walls of *Brachypodium distachyon* and generate a saturated EMS mutant population for forward genetic studies in this model bioenergy crop.

Computational Resources for Biofuel Feedstock Species

- C. Robin Buell, Michigan State University

Goal: Provide computational tools and resources for data-mining of genome sequence, genome annotation, and large-scale functional genomic datasets available for biofuel feedstock species. Such species include candidates within the *Poaceae*, *Pinaceae*, and *Salicaceae* families, for which a diversity of genome sequence resources currently exist, ranging from whole-genome sequences to modest EST transcriptome datasets.

Translational Genomics for the Improvement of Switchgrass

- Nick Carpita, Purdue University

Goal: Study the cell walls of grass species, performing bioinformatics analyses on cell-wall biosynthetic genes in maize, and annotate switchgrass orthologs. The project also will generate mutants in selected candidate cell wall-related genes, with direct analysis of saccharification of maize and switchgrass cell-wall mutants.

Identification of Genes That Regulate Phosphate Acquisition and Plant Performance During Arbuscular Mycorrhizal Symbiosis in *Medicago truncatula* and *Brachypodium distachyon*

- Maria Harrison, Boyce Thompson Institute for Plant Research

Goal: Identify genes controlling arbuscular mycorrhizal symbiosis, as well as key factors regulating gene function and the acquisition of key nutrients such as phosphate. The results will provide mechanistic and molecular-level understanding of plant-fungal partnerships in natural ecosystems and their role in maintaining a terrestrial soil environment for sustainable biofuel production.

Systems-Level Engineering of Plant Cell-Wall Biosynthesis to Improve Biofuel Feedstock Quality

- Samuel Hazen, University of Massachusetts

Goal: Identify and characterize cell-wall biosynthetic regulatory genomic binding sites using reverse and forward genetic approaches with candidate transcription factors in *Brachypodium* and *Arabidopsis*, two model plant systems. The results will contribute to our understanding of key tissue-specific and developmental regulators of plant cell-wall biosynthesis in monocot and dicot bioenergy crops.

Genomics of Wood Formation and Cellulosic Biomass Traits in Sunflower

- Steven Knapp, University of Georgia

Goal: Develop genomic resources for woody biomass trait identification in hybrid sunflower, a species that is extremely drought tolerant. This fundamental knowledge will complement the existing body of work on this species with respect to oilseed production.

Identification of Genes that Control Biomass Production Using Rice

- Jan Leach, Colorado State University

Goal: Provide an integrated breeding and genomics platform to identify biomass traits in rice, for translation to second-generation bioenergy grasses such as switchgrass and *Miscanthus*.



Rice image courtesy of the USDA Agricultural Research Service.

A Universal Genome Array and Transcriptome Atlas for *Brachypodium distachyon*

- Todd Mockler, Oregon State University

Goal: Develop an Affymetrix genome tiling array, based on the DOE Joint Genome Institute sequence of *Brachypodium distachyon*, and make the array available for broad community use. The investigators will use the array to generate an expression atlas representing major developmental stages or stress responses in *Brachypodium*, a model species for polyploid, perennial grasses with complex genomes, such as wheat and switchgrass.

Epigenomics of Development in *Populus*

- Steven Strauss, Oregon State University

Goal: Construct a study of the role of chromatin modification (epigenetics) in the regulation of development and dormancy induction in poplar and other woody species. The investigators will characterize changes in DNA methylation patterns on specific tissues during dormancy induction and poplar development.

2007 Awards

Eleven Projects Selected for Awards Totaling \$8.3 Million

Towards a Map of the *Populus* Biomass Protein-Protein Interaction Network

- Eric Beers, Virginia Tech

Goal: Map protein-protein interactions relevant to biomass production by focusing on proteins coexpressed in poplar xylem, site of the majority of lignocellulose synthesis and hence biomass accumulation in poplar.

Strategies for Using Molecular Markers to Simultaneously Improve Corn Grain Yield and Stover Quality for Ethanol Production

- Rex Bernardo, University of Minnesota

Goal: Optimize the use of DNA markers to simultaneously breed for high corn grain yield (for nonenergy and energy uses) and high stover quality for ethanol production.

Developing Association Mapping in Polyploid Perennial Biofuel Grasses

- Ed Buckler, USDA Agricultural Research Service

Goal: Undertake an association-mapping study of two important biofuel grasses, switchgrass and reed canarygrass, to identify molecular markers tightly linked to biomass-related trait loci. This will enable marker-assisted selection and greatly accelerate breeding programs for enhanced biomass production.

Resource Development in Switchgrass, an Important Bioenergy Crop for the U.S.A.

- Katrien Devos, University of Georgia

Goal: Construct a detailed genetic map of switchgrass based on simple sequence repeats and align it with maps produced in rice, maize, and sorghum. This will allow the exploitation of resources and sequence information generated for these well-studied cereals. The genetic maps also will serve as a framework for locating genes that control bioenergy traits.

Development of Genomic Tools to Improve Prairie Cordgrass (*Spartina pectinata*), a Highly Productive Bioenergy Feedstock Crop

- Jose Gonzalez, South Dakota State University

Goal: Develop PCR markers for this species and construct an initial linkage map for prairie cordgrass, a native perennial high-biomass yielding grass.

Analysis of Small RNAs and mRNAs Associated with Abiotic Stress Responses in *Brachypodium distachyon*

- Pam Green, University of Delaware

Goal: Identify small RNAs related to stresses such as drought, temperature, and nutrient deprivation and relate them to the emerging genome sequence of *Brachypodium distachyon*, thus enhancing its value as a functional genomic model for energy crops and temperate grasses.

Identification of Cell-Wall Synthesis Regulatory Genes Controlling Biomass Characteristics and Yield in Rice (*Oryza sativa*)

- Zhaohua Peng, Mississippi State University

Goal: Examine cell-wall synthesis in rice, a model grass bioenergy species and the source of rice stover residues, using reverse genetic and functional genomic and proteomic approaches.

Linkage Analysis Appropriate for Comparative Genome Analysis and Trait Selection in Switchgrass

- Christian Tobias, USDA Agricultural Research Service

Goal: Create a comprehensive marker set for switchgrass based principally on simple sequence repeats, and initiate development of a linkage map.

A Functional Genomics Approach to Altering Crown Architecture in *Populus*: Maximizing Carbon Capture in Trees Grown in Dense Plantings

- Jerry Tuskan, Oak Ridge National Laboratory

Goal: Gain a molecular understanding of phytochrome-mediated responses to competition in *Populus* and then use that knowledge to maximize carbon capture per unit of land area for increased biomass production.

Development of Brown Midrib Sweet Sorghum as a Dual-Source Feedstock for Ethanol Production

- Wilfred Vermerris, University of Florida

Goal: Maximize the amount of fermentable sugar in the whole sorghum plant by identifying and isolating genes that control the high stalk juice sugar trait and a decreased stalk lignin trait, with the aim of eventually combining both traits in a single germplasm.

Insertional Mutagenesis of *Brachypodium distachyon*

- John Vogel, USDA Agricultural Research Service

Goal: Create a collection of insertional mutants in *Brachypodium distachyon*. This resource collection then can be used to identify mutations in genes predicted to affect biomass quality and agronomic characteristics of other perennial grass energy crops.



Brachypodium distachyon
image courtesy of Oregon State University.

2006 Awards

Nine Projects Selected for Awards Totaling \$5.7 Million

Using Association Mapping to Identify Markers for Cell Wall Constituents and Biomass Yield in Alfalfa

- Charles Brummer, University of Georgia

Goal: Use genomics approaches to identify chromosomal regions, and ultimately genes, controlling the two most important bioenergy traits, biomass yield and composition, and develop genetic markers that can be used directly in applied plant breeding programs to improve the bioenergy qualities of alfalfa.

Manipulation of Lignin Biosynthesis to Maximize Ethanol Production from *Populus* Feedstocks

- Clint Chapple, Purdue University

Goal: Generate transgenic poplar up- or down-regulated for four enzymes known to impact lignin quantity and quality; develop metabolic profiling methods for poplar and their application to greenhouse- and field-grown wild-type and transgenic plants; conduct morphometric analysis of transgenic lines grown in field plots; and analyze cell-wall deconstruction for wild-type and lignin-modified transgenic lines.

Genomic Knowledgebase for Facilitating the Use of Woody Biomass for Fuel Ethanol Production

- Vincent Chiang, North Carolina State University

Goal: Establish a knowledgebase about the possible genes and transcription factor genes involved in lignocellulosic formation and those genes that may enable effective manipulation of lignocellulosic traits to facilitate ethanol production.

Systematic Modification of Monolignol Pathway Gene Expression for Improved Lignocellulose Utilization

- Richard Dixon, The Noble Foundation

Goal: Determine which features of the lignocellulosic material (lignin content, lignin composition, or other factors) are most detrimental to the fermentation of biomass to ethanol and develop the crop plant alfalfa (*Medicago sativa*) as a model system for genomic studies on biomass utilization.

Genetic Dissection of the Lignocellulosic Pathway of Wheat to Improve Biomass Quality of Grasses as a Feedstock for Biofuels

- Bikram Gill, Kansas State University

Goal: Investigate the expression of ~80 candidate genes for lignin biosynthesis, their enzymatic activities, and lignin content and composition in different organs at different

stages of diploid wheat plant; silence these 80 genes individually by VIGS; identify knockout mutants of these genes using TILLING; and characterize the silenced tissues and knockout mutants by metabolite profiling.

Biochemical Genomics of Wood Formation: O-Acyltransferases for Alteration of Lignocellulosic Property and Enhancement of Carbon Deposition in Poplar

- Chang-Jun Liu, Brookhaven National Laboratory

Goal: Identify genome-wide acyl-CoA dependent acyltransferase genes from poplar genomics database; systemically explore the tissue-specific and stress-responsible expression patterns of O-acyltransferase genes to identify the enzymes specifically involved in lignocellulosic biosynthesis; and systemically characterize the biochemical functions of acyltransferases responsible for polysaccharide acetylation, lignol biosynthesis, and phenolic compound modification.

Streamlined Method for Biomass Whole-Cell-Wall Structural Profiling

- John Ralph, USDA Agricultural Research Service, University of Wisconsin

Goal: Provide the plant cell-wall and biomass research communities with improved methods for polysaccharide and lignin structural profiling, based on complete cell-wall solubilization and NMR. The aim is to develop and streamline procedures to allow 20 to 30 samples per day to be profiled.

Sorghum Biomass/Feedstock Genomics Research for Bioenergy

- William Rooney, Texas A&M University

Goal: Annotate genes, pathways, and regulatory networks identified in the sorghum genome sequence that are important for biomass generation, and identify, map, and clarify the function of trait loci that modulate accumulation and quality of biomass in sorghum.

Development of a Proteoglycan Chip for Plant Glycomics

- Chris Somerville, Carnegie Institute of Washington

Goal: Develop high-throughput methods and reagents that will facilitate the assignment of function to large numbers of glycosyltransferases and other glycan-modifying enzymes.

