

Plant Feedstock Genomics for Bioenergy Joint Awards 2006–2015

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Integrating the U.S. Department of Energy's (DOE) capabilities in genomic sequencing and analysis for biofuels production 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. 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 USDA's National Institute of Food and Agriculture. This program supports fundamental biomass genomics research to provide the scientific foundation for the use of lignocellulosic and seed oil materials, either primary material or agricultural residues, for bioenergy and biofuels.

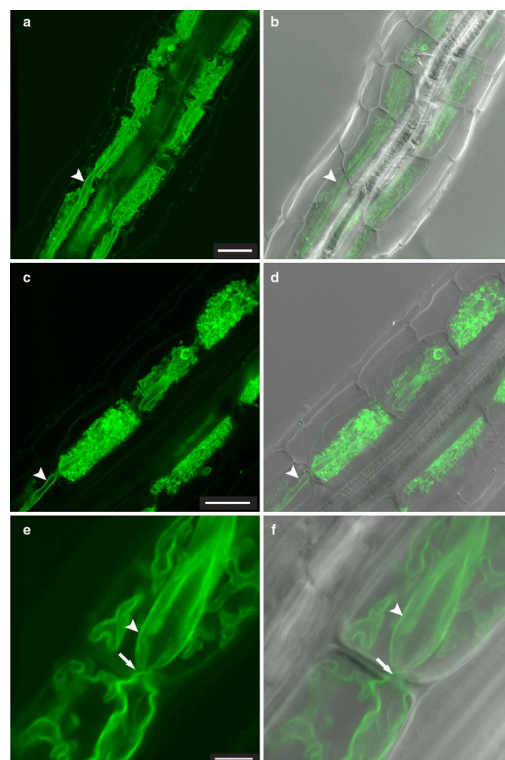
Significant advances in breeding, molecular genetics, and genomic technologies offer an opportunity to build upon the existing plant biology knowledgebase to be able to confidently predict and manipulate the biological function of plant genomes for bioenergy resources. This year, the program seeks to enhance these genetic and genomic resources by supporting research to investigate potential challenges likely to be encountered with increasing acreage devoted to bioenergy feedstocks, specifically the molecular mechanisms underlying plant resistance and tolerance to pathogens. This knowledge will be used to accelerate breeding of improved dedicated bioenergy feedstocks. Areas of interest include:

- Genomics-based research to identify and functionally characterize key plant genes/alleles that confer disease resistance/tolerance.

- Research to develop new cultivars of regionally adapted energy feedstocks with enhanced biomass yield and resistance/tolerance to pathogens.

Project investigators are characterizing the genes, proteins, and molecular interactions that influence plant response to pathogens with a focus on crop improvement. The long-term goal is to develop a clearer understanding of the key genes and processes responsible for superior feedstock plant performance under increasing pathogen pressure and with minimal impacts on the surrounding ecological landscape. The results will be used to develop improved, high-performing lignocellulosic biomass and oilseed crops for bioenergy.

Colonization patterns of three arbuscular mycorrhizal fungi in *Brachypodium distachyon*. Shown are *B. distachyon* roots colonized with (a–b) *Gigaspora gigantea*, (c–d) *Glomus intraradices*, and (e–f) *G. versiforme*. Roots are stained with WGA-alexafuor 488 to reveal fungal structures (green). Arrowheads point to intracellular hyphae. Arrows point to hyphal constrictions where the fungal hyphae exit and enter cells. [a–d, bars: 20 µm; e–f, bar: 5 µm; panels a, c, and e: fluorescence images; panels b, d, and f: fluorescence images overlaid on bright field (differential interference contrast images)]



[a–d, bars: 20 µm; e–f, bar: 5 µm; panels a, c, and e: fluorescence images; panels b, d, and f: fluorescence images overlaid on bright field (differential interference contrast images)] Courtesy Boyce Thompson Institute for Plant Research. Hong, J. J., et al. 2012. "Diversity of Morphology and Function in Arbuscular Mycorrhizal Symbioses in *Brachypodium distachyon*," *Planta* 236, 851–65.)

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Cover photograph courtesy University of Georgia, Athens

More details including Co-PIs and funding amounts are at genomicscience.energy.gov/research/DOEUSDA/.

2015 Awards

Five Projects Selected for Awards Totaling \$4.9 Million

Physiological and Molecular-Genetic Characterization of Basal Resistance in Sorghum

- Peter Balint-Kurti, North Carolina State University, Raleigh

Goal: Identify loci and alleles that will aid breeders in producing more robust sorghum lines designed for biomass production. This project will develop assays and use them to screen diverse sorghum germplasm for variation in defense response and disease resistance and to identify genes associated with this variation. The resulting findings will be used to develop quantitative, durable disease resistance for improved bioenergy sorghum.



Sorghum lines infected with *Bipolaris cookei*, which causes target leaf spot. The row on right is susceptible; row on left is resistant. (Courtesy Peter Balint-Kurti, North Carolina State University, Raleigh)

Genomic Dissection of Anthracnose Resistance Response in Sorghum [*Sorghum bicolor* (L.) Moench]

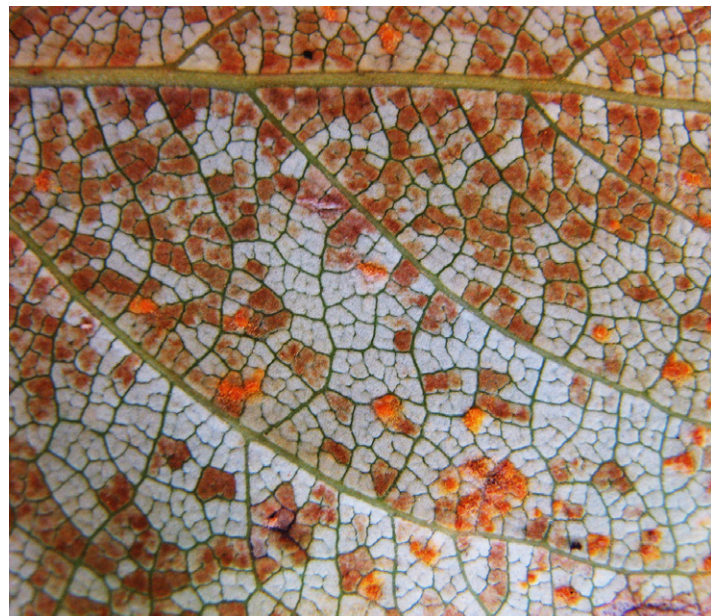
- Hugo Cuevas, USDA Agricultural Research Service, Mayaguez, Puerto Rico

Goal: Identify anthracnose resistance loci from diverse sorghum germplasm, establish the pathotypes against which the resistance alleles at these loci protect, and determine the underlying disease resistance mechanism. This work will enable dissection of the anthracnose resistance response into its multiple gene components and further understanding of the host-pathogen relationship present in different sorghum types. Results will accelerate breeding and provide plant breeders with a tool kit that provides maximum resistance levels in the intended production area.

Characterizing the Defense Hierarchy of *Populus trichocarpa* and Its Hybrids

- George Newcombe, University of Idaho, Moscow

Goal: Develop an integrative, hierarchical model of *P. trichocarpa* defense that integrates genetic resistance and defense mutualists. This study will test the placement of several factors that contribute to rust resistance under different circumstances, including major and minor plant resistance genes, plant defense compounds, direct competitors, and defense mutualists within the microbiome. The goal is to develop disease management strategies that harness both resistance genes and naturally occurring defense mutualists of *P. trichocarpa*, maximizing plant resistance and productivity while minimizing impacts on the surrounding ecological landscape.



Rust and bronzing patchwork on leaves of *Populus trichocarpa* is typical in poplar west of the Cascades. (Courtesy George Newcombe, University of Idaho, Moscow)

Genomics-Assisted Breeding for Leaf Rust (*Melampsora*) Resistance in Shrub Willow (*Salix*) Bioenergy Crops

- Larry Smart, Cornell University, Geneva, NY

Goal: Identify genes that are involved in rust resistance in willow that then can be introgressed into new, improved willow cultivars through hybridization. This study also will generate molecular markers linked to rust-resistance genes for use in the early selection of resistant seedlings in breeding programs. The ultimate goal is to develop improved, rust-resistant willow cultivars with greater yields, thus encouraging wider adoption of willow for renewable energy (see image next page).



Symptoms of *Melampsora* leaf rust on a susceptible shrub willow cultivar (left; also see inset) growing adjacent to a resistant cultivar. (Courtesy Larry Smart, Cornell University, Geneva, NY)

Parallel Analysis of *Puccinia emaculata* Virulence and Switchgrass Resistance Phenotypes

- Shavannor Smith, University of Georgia, Athens

Goal: Identify candidate effector genes in *P. emaculata* that interact with specific switchgrass resistance genes, and develop and test models of these interactions on switchgrass cultivars infected with field rust isolates. This study will reveal new strategies for generating more durable resistance to *P. emaculata* and other pathogens. Results will provide the knowledgebase to develop diagnostic tools for rapidly assessing the nature of a field *P. emaculata* isolate, thus identifying host resistance cultivars that exhibit optimal resistance to field pathogen populations at any given location.



Field plot of *P. emaculata* rust on susceptible and resistant switchgrass phenotypes. (Courtesy Shavannor Smith, University of Georgia, Athens; also on cover)

2014 Awards

Ten Projects Selected for Awards Totaling \$12.6 Million

Coordinated Genetic Improvement of Bioenergy *Sorghum* for Compositional and Agronomic Traits

- Patrick Brown, University of Illinois, Urbana-Champaign

Goal: Discover and characterize novel genetic variants that affect lignocellulosic composition and saccharification yield in bioenergy feedstock grasses without compromising agronomic performance. This project will characterize genetic variation and identify useful traits and variants that will guide and accelerate the genetic improvement of bioenergy *Sorghum* and closely related perennial grasses.

Abiotic Stress Networks Converging on FT2 to Control Growth in *Populus*

- Amy Brunner, Virginia Polytechnic Institute and State University, Blacksburg

Goal: Uncover regulatory networks that control growth and dormancy responses to daylength and nutrient stress in poplar. This project will characterize genome-wide gene expression changes in response to these environmental factors and identify protein-protein and protein-DNA networks that are centered on FT2, a key integrator of multiple abiotic signaling pathways in *Populus*.

Exploiting Natural Diversity to Identify Alleles and Mechanisms of Cold Adaptation in Switchgrass

- Robin Buell, Michigan State University, East Lansing

Goal: Identify metabolites, alleles, transcripts, and regulatory RNAs associated with cold hardiness in switchgrass to advance understanding of the biochemical, physiological, and molecular mechanisms for cold adaptation and provide molecular tools to increase biomass and extend the growing range of lowland ecotypes into northern latitudes.

A Novel Poplar Biomass Germplasm Resource for Functional Genomics and Breeding

- Luca Comai, University of California, Davis

Goal: Extend and use the poplar indel germplasm collection to investigate the role of gene dosage in poplar hybrid performance and contribution to bioenergy traits. This project will catalog dosage variation, characterize variation for traits central to sustainable production of high quality biomass, and identify specific regulatory or functional gene modules underlying phenotypes of interest.

Genetic Dissection of AM Symbiosis to Improve the Sustainability of Feedstock Production

- Maria Harrison, Boyce Thompson Institute for Plant Research, Ithaca, NY

Goal: Understand the genetic bases of arbuscular mycorrhizal (AM) symbiosis in feedstocks through studies of *Brachypodium distachyon* and *Sorghum*. This project will utilize the model species *Brachypodium* to evaluate the function of proteins that potentially control symbiosis development and then evaluate AM symbiosis in sweet and energy *Sorghum*.

Advancing Field Pennycress as a New Oilseed Biodiesel Feedstock That Does Not Require New Land Commitments

- Michael Marks, University of Minnesota, Minneapolis

Goal: Genetically improve field pennycress (*Thlaspi arvense* L.) for use as a new winter annual oilseed crop in the Upper Midwest that can be

double-cropped with corn and soybeans. This project will lead to superior, higher-yielding pennycress varieties grown as a winter oilseed crop integrated within corn-soybean rotations throughout the Midwest.

Biofuels in the Arid West: Germplasm Development for Sustainable Production of *Camelina* Oilseed

- John McKay, Colorado State University, Fort Collins

Goal: Facilitate the development of *Camelina* as an oilseed feedstock crop that can be grown on marginal farmland with relatively low fertilizer inputs and no irrigation. This project will use forward and reverse genetics and natural variation to combine optimal qualities in *Camelina* as an oilseed feedstock for the Great Plains and Western United States.



Camelina oilseed feedstock crops can be grown on marginal farmland (lower fertilizer inputs and no irrigation). (Courtesy Jean-Nicolas Enjalbert, Colorado State University)

The *Brachypodium* ENCODE Project—From Sequence to Function: Predicting Physiological Responses in Grasses to Facilitate Engineering of Biofuel Crops

- Todd Mockler, Donald Danforth Plant Science Center, St. Louis, MO

Goal: Identify and characterize the functional elements associated with progressive drought response in the *Brachypodium distachyon* genome sequence and develop integrated genome feature maps that enable advanced modeling of complex plant pathways. The *Brachypodium* ENCODE (for Encyclopedia of DNA Elements) project will elucidate the molecular mechanisms and gene regulatory networks underlying drought stress, accelerating deployment of improved bioenergy grass feedstocks.

Genomics of Energy Sorghum's Water Use Efficiency/Drought Resilience

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

Goal: Use field analyses to identify traits and molecular responses that improve water use efficiency and drought resilience of energy *Sorghum* and characterize genetic variation, and then test the utility of modulating these traits in energy *Sorghum* hybrids through marker-assisted breeding.

Quantifying Phenotypic and Genetic Diversity of *Miscanthus sacchariflorus* to Facilitate Knowledge of Directed Improvement of *M. × giganteus* (*M. sinensis* × *M. sacchariflorus*) and Sugarcane

- Erik Sacks, University of Illinois, Urbana-Champaign

Goal: Facilitate the rapid development of *Miscanthus* as a bioenergy crop by obtaining fundamental knowledge about *M. sacchariflorus* (*Msa*) genetic diversity, population structure, and environmental adaptation. This project will evaluate yield potential and adaptation of *Msa* from throughout its natural range, and develop molecular markers associated with traits of interest that will enable plant breeders to quickly develop improved biomass cultivars.

2013 Awards

Seven Projects Selected for Awards Totaling \$8.1 Million

Functional Manipulation of Root Endophyte Populations for Feedstock Improvement

- Jeffrey Dangl, University of North Carolina, Chapel Hill

Goal: Utilize genomics, genetics, and physiology to understand how endophytic bacteria alter plant growth and productivity, ultimately to manipulate plant performance for feedstock production. A variety of plant functions and traits are co-dependent on the surrounding microbial community, especially those associated with the plant root system (rhizosphere). This project will investigate whether plant performance phenotype in association with microbial communities translates across plant species in a predictable manner.

Unraveling the Genetics of Two Key Biomass Traits that Differentiate Upland and Lowland Tetraploid Switchgrass Ecotypes, Colonization by Mycorrhizal Fungi, and Frost Tolerance

- Katrien Devos, University of Georgia, Athens

Goal: Develop strategies for increased frost tolerance of lowland switchgrass through (1) identifying the genetic pathway(s) that provide frost tolerance in upland switchgrass and (2) studying the potential of beneficial fungi to minimize host cold stress. This project seeks to leverage the high biomass yield of southern-adapted lowland types and the frost tolerance of northern-adapted upland types to identify candidate genes that can be exploited to enhance biomass production of switchgrass under cold stress.

Genetic Control of Flowering in Switchgrass

- Yiwei Jiang, Purdue University, West Lafayette, IN

Goal: Elucidate the genetic mechanisms and identify candidate genes controlling flowering time in switchgrass. Late-flowering genotypes yield more biomass because the growing season is extended; having a better understanding of the genes that control flowering time will help to develop a rational strategy for creating improved switchgrass lines. The knowledge generated will aid breeding programs in developing late-flowering switchgrass varieties that fully utilize the growing season and achieve high biomass yield.

Accelerated Development of Optimal Pine Feedstocks for Bioenergy and Renewable Chemicals Using Genome-Wide Selection

- Matias Kirst, University of Florida, Gainesville

Goal: Hyper-accelerate pine breeding using genome-wide selection, generating cultivars of loblolly and slash pine tailored to produce high energy yields that are ready for deployment. Traditional genetic improvement of pines is logistically complex and expensive, and a single breeding cycle takes almost two decades to complete. Thus, the project will develop and apply new breeding strategies that accelerate development of cultivars suitable for bioenergy production.

Pyramiding Genes and Alleles for Improving Energy Cane Biomass Yield

- Ray Ming, University of Illinois, Urbana-Champaign

Goal: Accelerate energy cane breeding and maximize biomass yield by utilizing the extraordinary segregation of true F2 populations to select high biomass-yielding genotypes. Sugarcane cultivars are mostly derived from hybridization between domesticated and wild species followed by backcrossing to recover the high biomass yield and sugar content of the domestic parent while retaining stress tolerance from the wild. Because sugar content is not a limiting factor for energy cane, this project will introduce a new breeding paradigm for more efficient cultivar improvement.

Global Analysis of Epigenetic Regulation of Gene Expression in Response to Drought Stress in *Sorghum*

- A. S. N. Reddy, Colorado State University, Fort Collins

Goal: Investigate the impact of drought stress on epigenetic modifications and alternative splicing in *Sorghum*. Using recently developed high-throughput tools, this project will examine genome-wide changes in the chromatin landscape and patterns of alternative splicing in cultivars that are sensitive and tolerant to drought under normal conditions and in response to drought stress. Understanding how plants respond and adapt to drought stress at the molecular level will help in developing plants that can grow under water-limiting conditions.

Structural Polymorphisms as Causes of Heterosis in *Populus*

- Steven Strauss, Oregon State University, Corvallis

Goal: Characterize the extent of structural polymorphisms (SPs) between and within species of *Populus* that are used to produce wood and bioenergy and examine their relationship to growth, stress tolerance, and breeding efficiency. This project will study wild black cottonwoods and interspecies hybrids important in plantations in the Pacific Northwest United States and other parts of the world, with a focus on the extent to which SP assays could improve hybrid breeding compared to alternative approaches.



Field trial of a pine feedstocks population used in a genome-wide selection project. (Courtesy Matias Kirst, University of Florida, Gainesville)

2012 Awards

Nine Projects Selected for Awards Totaling \$11.5 Million

Functional Gene Discovery and Characterization of Genes and Alleles Affecting Wood Biomass Yield and Quality in *Populus*

- Victor Busov, Michigan Technological University, Houghton

Goal: Discover and characterize novel genes and alleles that affect wood biomass yield and quality in *Populus*. By combining mutagenesis for functional identification of genes with next-generation sequencing technologies for identifying alleles with breeding values, these discoveries can enable knowledge-based approaches for development of specialized bioenergy poplar cultivars.

Identifying Differences in Abiotic Stress Gene Networks Between Lowland and Upland Ecotypes of Switchgrass

- Kevin Childs, Michigan State University, East Lansing

Goal: Investigate response to drought and salt stress in a diverse collection of lowland and upland switchgrass ecotypes. Comparing differential gene expression between tolerant and sensitive lines will enable a better understanding of this response, as well as the identification of genes and germplasm that can be used to improve cultivated switchgrass to better tolerate these abiotic stresses.

Poplar Interactome for Bioenergy Research

- Pankaj Jaiswal, Oregon State University, Corvallis

Goal: Identify genome-wide functional gene networks and subnetworks in poplar that are associated with abiotic stress tolerance and bioenergy-related traits. Using a combination of computational projections, gene expression analysis, and experimental validation, this project will further development of poplar varieties that can thrive under abiotic stress on marginal land.

The Genetics of Biofuel Traits in *Panicum* Grasses: Developing a Model System with Diploid *Panicum hallii*

- Thomas Juenger, University of Texas, Austin

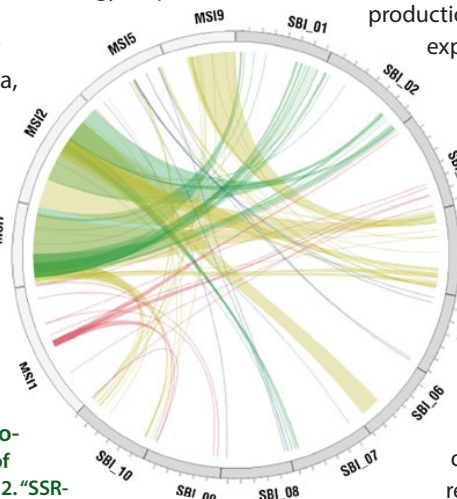
Goal: Investigate the growth and development of *Panicum* grasses and provide tools for predicting biomass- and tissue-related phenotypes from genotypes. This project will exploit natural variation to discover the genes important in biomass production, quality, and stress tolerance under diverse environmental conditions, providing avenues for improving C4 perennial grasses for use as bioenergy crops.

Genomics of Bioenergy Grass Architecture

- Andrew Paterson, University of Georgia, Athens

Goal: Understand the genetic determinants of plant architecture that are important to the design of sorghum genotypes optimized for biomass production in a range of environments. This project will further enhance the value of many existing resources while also adding new dimensions to scientific research capacity.

Linkage groups of *Miscanthus sinensis* (MSI) in a circle alignment comparing MSI chromosomes to Sorghum chromosomes. (Courtesy University of Georgia, Athens. Kim, C., et al. 2012. "SSR-Based Genetic Maps of *Miscanthus sinensis* and *M. sacchariflorus*, and Their Comparison to *Sorghum*," *Theoretical Applied Genetics* 124(7), 1325–38.)



Deciphering Natural Allelic Variation in Switchgrass for Biomass Yield and Quality Using a Nested Association Mapping Population

- Malay Saha, Samuel Roberts Noble Foundation, Ardmore, OK

Goal: Understand the genetic basis of key biofeedstock traits in switchgrass by identifying genetic markers controlling important, complex biomass traits such as biomass yield and cell wall composition. Validated markers cosegregating with bioenergy-relevant traits will be used to initiate a marker-assisted and/or genomic selection program to accelerate development of superior cultivars.

Genetic Architecture of Sorghum Biomass Yield Component Traits Identified Using High-Throughput, Field-Based Phenotyping Technologies

- Patrick Schnable, Iowa State University, Ames

Goal: Test the hypothesis that variation in biomass growth rate can be explained by variation in photosynthetic rates and/or amounts of photo-protection. Sorghum data collected at multiple time points using an automated high-throughput field-based plant phenotyping system will be analyzed to identify the genetic control of growth rate, allowing breeders to genetically "stack" relevant genes and produce higher-yielding hybrids.



New field-based technology will digitize and screen for biomass yield traits. The converted John Deere tractor "robot" has a GPS-enabled auto-steer function and sensors. Researchers "train" its function to follow a specific route. (Courtesy Patrick Schnable, Iowa State University, Ames)

The Genomic Basis of Heterosis in High-Yielding Triploid Hybrids of Willow (*Salix* spp.) Bioenergy Crops

- Lawrence Smart, Cornell University, Ithaca, NY

Goal: Investigate how gene expression patterns in willow hybrids are related to yield potential and other traits important for biofuels production. This project will study whether there is a bias in the expression of key genes from one hybrid parent versus the other and whether gene dosage skews expression patterns in triploid progeny compared with their diploid and tetraploid parents.

The Dual Effect of Tubulin Manipulation on *Populus* Wood Formation and Drought Tolerance

- Chung-Jui Tsai, University of Georgia, Athens

Goal: Determine how tubulin levels and/or tubulin protein modifications affect wood development and water use in *Populus*. This project will dissect tubulin contribution to two interdependent processes, water use and development of lignocellulosic biomass, which are relevant to bioenergy crop improvement.

2011 Awards

Ten Projects Selected for Awards Totaling \$12.2 Million

Association Mapping of Cell Wall Synthesis Regulatory Genes and Cell Wall Quality in Switchgrass

- Laura E. Bartley, University of Oklahoma, Norman

Goal: Identify natural genetic variation in switchgrass that correlates with lignocellulose-to-biofuel conversion qualities. Most plant dry matter is composed of lignocellulose. Because switchgrass yields high amounts of this material and tolerates drought and other stresses, it is an attractive candidate for development into a biofuel crop. This project should enhance understanding of the qualities that critically impact the conversion efficiency of lignocellulose into biofuels.

Functional Interactomics: Determining the Roles Played by Members of the Poplar Biomass Protein-Protein Interactome

- Eric Beers, Virginia Polytechnic and State University, Blacksburg

Goal: Identify key interactions between proteins associated with wood formation in poplar, a woody biomass crop. Wood characteristics result from the coordinated actions of enzymes and structural proteins in the cells, which typically interact with other proteins to perform their roles. This project will uncover the potential of the biomass protein-protein interactome to contribute to the development of poplar trees with superior biomass feedstock potential.

Functional Genomics of Sugar Content in Sweet *Sorghum* Stems

- David M. Braun, University of Missouri, Columbia

Goal: Improve sucrose accumulation in sweet *Sorghum* by investigating the mechanisms regulating carbon allocation to stems. A rapidly growing, widely adaptable crop, sweet *Sorghum* accumulates in the stem high concentrations of sucrose that can be efficiently converted to ethanol, making this a valuable bioenergy feedstock candidate. This research will use a combination of approaches to identify sweet *Sorghum*'s bioenergy-relevant genes and understand their function in carbon partitioning.

Creation and High-Precision Characterization of Novel *Populus* Biomass Germplasm

- Luca Comai, University of California, Davis

Goal: Provide new genomic tools for poplar breeders to identify germplasm with unique genotypes and increased biomass yields and develop techniques for creating poplar hybrids with unique combinations of chromosomal regions. Because such properties can confer faster growth, this project addresses a challenge posed by the long generation time of trees through fast and cost-effective nontransgenic genetic manipulation.

Genomic and Breeding Foundations for Bioenergy *Sorghum* Hybrids

- Stephen Kresovich, University of South Carolina, Columbia

Goal: Build the germplasm, breeding, genetic, and genomic foundations necessary to optimize cellulosic *Sorghum* as a bioenergy feedstock. This project will facilitate breeding *Sorghum* lines optimized for energy production and selected to maximize energy accumulation per unit time, land area, and/or production input.

An Integrated Approach to Improving Plant Biomass Production

- Jan Leach, Colorado State University, Fort Collins

Goal: Expedite the discovery of genes controlling biomass productivity in switchgrass by leveraging results from rice, a well-studied model grass. Switchgrass and other perennial grasses are promising bioenergy feedstock candidates; however, the genetic resources necessary to develop these species are limited. This work will greatly expand the research tool box for switchgrass and advance its improvement as an energy crop.

Modulation of Phytochrome Signaling Networks for Improved Biomass Accumulation Using a Bioenergy Crop Model

- Todd C. Mockler, Donald Danforth Plant Science Center, St. Louis, MO

Goal: Identify genes involved in light perception and signaling in the model grass *Brachypodium distachyon* to increase yield and improve the composition of bioenergy grasses. Plant growth and development, including biomass accumulation, are affected by the light environment. Finding key genes involved in modulating light perception could be useful in targeted breeding or engineering efforts for improved bioenergy grass crops.

Quantifying Phenotypic and Genetic Diversity of *Miscanthus sinensis* as a Resource for Knowledge-Based Improvement of *M. × giganteus* (*M. sinensis* × *M. sacchariflorus*)

- Erik J. Sacks, University of Illinois, Urbana-Champaign

Goal: Facilitate development of *Miscanthus* as a bioenergy crop by obtaining fundamental information about its genetic diversity and environmental adaptation. *Miscanthus* is among the most promising cellulosic biofuel crops, but its improvement as a feedstock will require a broader genetic base. Identification of molecular markers associated with traits of interest will improve *Miscanthus* breeding efforts.

Discovering the Desirable Alleles Contributing to the Lignocellulosic Biomass Traits in *Saccharum* Germplasm Collections for Energy Cane Improvement

- Jianping Wang, University of Florida, Gainesville

Goal: Improve energy cane by identifying the genetic components contributing to biomass production. Energy cane (*Saccharum* complex hybrids) holds great potential as a bioenergy feedstock in the southern United States. This project will produce foundational genetic resources for energy cane breeders to efficiently develop cultivars with increased biomass production and reduced input requirements.

Sorghum Biomass Genomics and Phenomics

- Jianming Yu, Kansas State University, Manhattan

Goal: Integrate key genomics-assisted approaches into biomass *Sorghum* research and combine them with high-throughput and traditional field-based phenotyping methods to enable advanced breeding strategies. Exploiting genetic diversity and understanding the genotype-phenotype relationship can lead to predictive approaches for efficient and cost-effective breeding.

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, 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, Danville, VA

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, Urbana-Champaign

Goals: Determine whether changes in the Glossy15 gene system of *Sorghum* might contribute to current physiological differences among grain and 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 corn plant material 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

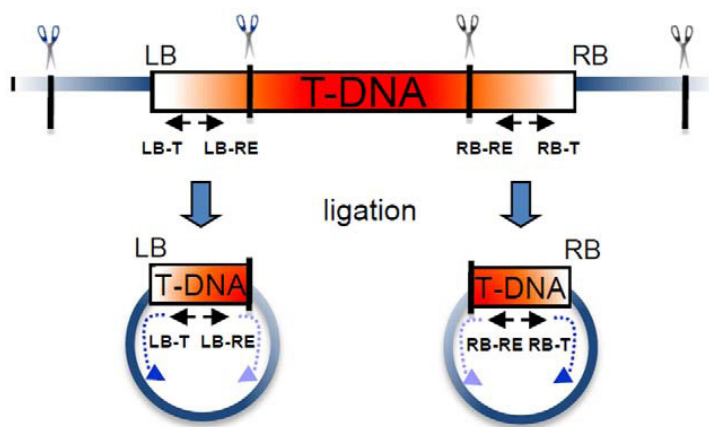
- Gary Stacey, University of Missouri, Columbia

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. 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 Vogel, USDA Agricultural Research Service, Albany, CA

Goals: Generate 30,000 additional insertional mutants in the model grass *Brachypodium distachyon* and sequence the DNA flanking the insertion sites. Insertional mutants are powerful research tools 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.



Inverse PCR strategy for obtaining T-DNA flanking sequences within *Brachypodium* genome sequence. (Courtesy USDA Agricultural Research Service, Albany, CA. Bragg, J. N., et al. 2012. “Generation and Characterization of the Western Regional Research Center,” *PLoS ONE* 7(9), e41916.)

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 biofuel-ready alfalfa cultivars with improved yield and quality. Maximizing energy production with biofuel crops requires high biomass yield with optimum fuel quality. This project will identify molecular markers in alfalfa that are associated with optimal biofuel characteristics and directly integrate them into traditional field-oriented alfalfa breeding programs.

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

- Victor Busov, Michigan Technological University, Houghton

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.

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*.

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

- Matias Kirst, University of Florida, Gainesville

Goal: Characterize genes that regulate the balance of carbon going to cellulose or lignin, leading to the development of plant materials 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, Lincoln, NE

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. This project will make a significant and lasting contribution to the future improvement of switchgrass as a bioenergy crop. It also will directly benefit researchers working on developing other perennial grasses into biomass energy crops.

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

- John Vogel, USDA Agricultural Research Service, Albany, CA

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 underlying biological traits that control the utility of grasses as energy crops.



Populus samples. Processed *Populus deltooides* ground samples ready for bioprocessing, unprocessed segments, and live *Populus* seedlings in the background. (Image courtesy Oak Ridge National Laboratory)

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, Athens

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's 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, University Park

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, East Lansing

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, West Lafayette, IN

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, Ithaca, NY

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, Amherst

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 the 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, Athens

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, Fort Collins

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*.

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

- Todd Mockler, Oregon State University, Corvallis

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. 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, Corvallis

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. This project will characterize changes in DNA methylation patterns on specific tissues during dormancy induction and poplar development.



Rice, a potential bioenergy grass. (Courtesy USDA Agricultural Research Service)

*SNP: single-nucleotide polymorphism; BAC: bacterial artificial chromosome; QTL: quantitative trait loci; EMS: ethylmethane sulfonate; EST: expressed sequence tag

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 Polytechnic and State University, Blacksburg

Goal: Map protein-protein interactions relevant to biomass production by focusing on proteins coexpressed in poplar xylem, the site of most 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, Minneapolis-St. Paul

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, Ithaca, NY

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 research 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, Athens

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 comparison will enable 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, Brookings

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, Newark

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, Mississippi State

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, Albany, CA

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, Oak Ridge, TN

Goal: Gain a molecular understanding of phytochrome-mediated responses to competition in *Populus* and use this 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, Gainesville

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, Albany, CA

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. (Courtesy Oregon State University)

*PCR: polymerase chain reaction

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, Athens

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

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

- Clint Chapple, Purdue University, West Lafayette, IN

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, Raleigh

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 Samuel Roberts Noble Foundation, Ardmore, OK

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, Manhattan

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, Upton, NY

Goal: Identify genome-wide acyl-CoA dependent acyltransferase genes from the 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, Madison, WI

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 enable profiling of 20 to 30 samples per day.*

Sorghum Biomass/Feedstock Genomics Research for Bioenergy

- William Rooney, Texas A&M University, College Station

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, Stanford, CA

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

*VIGS: virus-induced gene silencing; TILLING: Targeting Induced Local Lesions IN Genomes; CoA: coenzyme A; NMR: nuclear magnetic resonance

Three-year-old growth of shrub willow in a genetic selection trial at Cornell University (Ph.D. student Fred Gouker holding height pole with markings every 2 feet). (Courtesy Larry Smart, Cornell University, Geneva, NY) Can just use blue sky color swatch to match cover.



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