5th Pan American
Plants and BioEnergy Meeting

Sante Fe, NM
August 4-7, 2016

5th Pan-American Congress on Plants and BioEnergy Program Booklet

Congress Program

Santa Fe Community Convention Center

Nambe Room – 2nd Floor

Thursday, August 4th, 2016

Start Time	End Time	Event
3:00 PM	7:15 PM	Registration Open and Poster Set-Up
7:15 PM	7:30 PM	Opening Remarks: Richard Sayre, LANL – Nambe Room
7:30 PM	8:30 PM	Opening Keynote Lecture: Maureen McCann, Purdue University
8:45 PM	9:30 PM	Poster Sessions – Pojoaque and OhKay Rooms

Friday, August 5th, 2016

Start Time	End Time	Event
7:00 AM	8:00 AM	Coffee and Pastries
7:00 AM	3:00 PM	Registration Open
8:30 AM	10:30 AM	**Engineering Plants and Algae for Enhanced Biomass Yield** Chair: Kim Ogden, University of Arizona
		8:30 AM – 9:00 AM John Mullet, TAMU *Crop Modeling, Genomic Analysis, Selection and Design of Energy Sorghum*
		9:00 AM – 9:30 AM Richard Sayre, LANL/MNC *Improving Photosynthetic Efficiency in Plants and Algae*
		9:30 AM – 10:00 AM Andrew Groover, USDA Forest Service, University of California, Davis *Systems Biology of Tension Wood Formation*
		10:00 AM – 10:30 AM Maureen Hanson, Cornell University *Enhancing the Efficiency of Photosynthesis in C3 Plants*
10:30 AM	11:00 AM	Coffee Break
11:00 AM	12:30 PM	**Engineering Plants and Algae for Enhanced Biomass Yield** Chair: John Mullet, TAMU
		11:00 AM – 11:30 AM Kim Ogden, University of Arizona *Algal Cultivation Strategies for Advanced Biomass Yield*
		11:30 AM – 12:00 PM Jamey Young, Vanderbilt *Modeling Carbon Flux in Photosynthesis*
		12:00 PM – 12:30 PM Adriana Pacheco Moscoa, Tecnológico de Monterrey *Transcriptome response to high CO2 concentrations of an environmental microalgae after eight years of enrichment: implications in carbon capture and byproduct generation*

12:30 PM	1:30 PM	Lunch – on your own
1:30 PM	3:00 PM	**Ligno-Cellulose Structure, Synthesis and Engineering** Chair: Marcos Buckeridge, University of São Paulo
		1:30 PM – 2:00 PM Sandrasegaram Gnanakaran, LANL *Computational studies of components of plant cell wall and thylakoid membrane*
		2:00 PM – 2:30 PM Breeanna Urbanowicz, University of Georgia *Elucidating the Mechanism of Xylan Biosynthesis: From Genes to Function*
		2:30 PM – 3:00 PM Candace Haigler, North Carolina State University *New insights into the structure and function of plant cellulose synthase and the cellulose synthesis complex*
3:00 PM	3:30 PM	Tea Break
3:30 PM	5:30 PM	**Ligno-Cellulose Structure, Synthesis and Engineering** Chair: Nick Carpita, Purdue University
		3:30 PM – 4:00 PM Phillip Rushton, Purdue University *Modeling Molecular Structure of a Cellulose Synthase Plant Conserved Region with X-ray Crystallography and Small Angle X-ray Scattering Illustrates Potential Functions*
		4:00 PM – 4:30 PM Marcos Buckeridge, University of São Paulo *Cell Wall Metabolism and Biomass of Sugarcane May Change Under Different Stressing Conditions Related to the Global Climate Change*
		4:30 PM – 5:00 PM Peter Ciesielski, NREL *Mesoscale modeling of coupled transport phenomena and conversion kinetics in plant cell walls and biomass particles*
		5:00 PM – 5:30 PM Alex Yi-Lin Tsai, Joint BioEnergy Institute *Feruloyl transferases AT5, AT9 and AT10 in the BAHD family are involved in cell wall biosynthesis in grasses*
5:30 PM	7:00 PM	Poster Sessions –
7:30 PM	8:45 PM	Dinner with Featured Keynote Lecture Todd Anderson, Department of Energy

Saturday, August 6th, 2016

Start Time	**End Time**	**Event**
8:00 AM	9:00 AM	Coffee and Pastries
8:30 AM	10:30 AM	**Next Generation Bioenergy Feedstocks *and* Bioproducts, Life Cycle Analysis and Sustainability of Biofuel Systems** Chair: Omar Holguin, NMSU
		8:30 AM – 9:00 AM Chris Staiger, Purdue University *Myosins XI are involved in trafficking of cellulose synthase complexes in Arabidopsis*

		9:00 AM – 9:30 AM Nate McDowell, LANL *Impact of Climate Change on Forest Ecosystems*
		9:30 AM – 10:00 AM Jason Quinn, Colorado State University *Techno-economic and Life Cycle Assessment: Integration of Experimental Systems with Engineering Process Modeling*
		10:00 AM – 10:30 AM Clint Chapple, Purdue University *The role of the Mediator complex in the regulation of lignin deposition*
10:00 AM	10:30 AM	Coffee Break
10:30 AM	12:30 PM	**Bioproducts and Lignocellulosic Biomass; Biomass Processing and Modeling** Chair: Daniel Bush, Colorado State University
		10:30 AM – 11:00 AM John Gordon, LANL *Chemical Conversion of Carbohydrates to Alkanes*
		11:00 AM – 11:30 AM Steven Thomas, Department of Energy-BETO *Regional Feedstock Partnership Data Underpin Yield Assumptions in the 2016 Billion Ton Update*
		11:30 AM – 12:00 PM Omar Holguin, NMSU *The Green Marathon: Algal Cultivation, Crop Protection, Co-products and Conversion as NMSU*
		12:00 PM – 12:30 PM Heather Coleman, Syracuse University *RNAi suppression of lignin biosynthetic genes in sugarcane improves glucose release without impacting sucrose production*
12:30 PM	1:30 PM	Lunch – on your own
1:30 PM	5:00 PM	Afternoon free to enjoy beautiful Santa Fe
5:00 PM	6:30 PM	Poster Viewing
6:45 PM	8:00 PM	Closing Party Buffet Dinner at the Outside Terrace of the Santa Fe Community Convention Center

Sunday, August 7th, 2016

Start Time	**End Time**	**Event**
8:00 AM	9:00 AM	Coffee and Pastries
9:00 AM	10:30 AM	**Biomass and Biofuel Systems Innovations** Chair: Richard Sayre, LANL/MNC
		9:00 AM – 9:30 AM Brent Shanks, Iowa State University *Intergrating Biology and Chemistry for the Production of Bioproducts*
		9:30 AM – 10:00 AM Daniel Bush, Colorado State University *Rice AP2/ERF transcription factor overexpression increases biomass accumulation and grain yield while simultaneously enhancing*

		tolerance to abiotic stress
		10:00 AM – 10:30 AM Sangeeta Negi, New Mexico Consortium *Phototropin; A Master Regulatory Control Gene that Controls Photosynthesis and Growth Processes in Chlamydomonas*
		10:30 AM – 11:00 AM Closing Remarks

Speaker Abstracts

Friday, August 5th, 2016, 8:30 AM - 9:00 AM
Crop Modeling, Genomic Analysis, Selection and Design of Energy Sorghum
John Mullet, TAMU
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Sandra Truong, Texas A&M University; Ryan McCormick, Texas A&M University; William Rooney, Texas A&M University

First generation energy sorghum hybrids have the high biomass yield potential needed for production of cost competitive biofuels and bio-products. The high biomass yield of hybrids is due to long vegetative growth duration, highly efficient radiation interception, good radiation use efficiency due in part to C4 photosynthesis, and elevated partitioning of biomass to stems. Energy sorghum is drought and heat resilient therefore the crop can capture and use intermittent rainfall for biomass production during its long growing season. Energy sorghum is therefore ideally suited for production on annual cropland that is marginal for food crops. Genetic improvement of the major grain crops since 1950 has been remarkably successful through annual yield improvements of 1-2%/year achieved by optimizing crop management, building large scale breeding programs, and using multilocation testing. Since energy sorghum is a new crop with a small scale breeding program, we sought to accelerate genetic improvement using crop-trait modeling, automated phenotyping, molecular genomic analyses, and QTL-based genomic selection technologies. Crop-trait modeling is being used to identify plant architectures (i.e., leaf angle) and physiological responses (i.e., deep rooting in response to water deficit) that have good potential for increasing the biomass yield or resilience of energy sorghum. Automated phenotyping systems, funded in part by the ARPA-E program TERRA, are being designed and deployed in controlled environments and in the field to improve the throughput and precision of canopy, plant, and trait phenotyping. Genetic analysis of populations derived from diverse germplasm is aiding the discovery of QTL, genes, and gene regulatory networks that modulate trait expression. This information is being used for genetic selection in the breeding program, and integrated with results from molecular genomic analyses to identify and engineer improved trait designs that could further enhance yield in energy sorghum's target region of production.

Friday, August 5th, 2016, 9:00 AM - 9:30 AM
Improving Photosynthetic Efficiency in Plants and Algae
Richard Sayre, LANL/MNC
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One of the more environmentally sustainable ways to produce high energy density (oils) liquid transportation fuels is photosynthetic reduction of carbon dioxide into carbohydrates and hydrocarbons and their subsequent conversion into fuels. Photosynthetic carbon capture from the atmosphere combined with bioenergy production (combustion) and subsequent carbon capture and sequestration

(BECCS) has also been proposed by the recent Intergovernmental Panel on Climate Change Report as the most effective and economical way to remediate atmospheric greenhouse gasses. To maximize carbon capture efficiency and energy-return-on-investment, we must develop cropping systems that have the greatest aerial biomass yields with the lowest inputs. All photosynthetic organisms, however, convert only a fraction (< 5%) of the solar energy they capture into harvestable chemical energy (reduced carbon or biomass). To increase aerial carbon capture rates and biomass productivity it will be necessary to increase photosynthetic efficiency in plants and algae. We will discuss metabolic engineering strategies to improve photosynthetic efficiency and biomass productivity in algal and plant systems, often borrowing metabolic strategies from one photosynthetic system to transfer into another. These strategies include optimization of photosynthetic light-harvesting antenna size and the introduction of algal inorganic carbon concentrating systems into plants to increase carbon fixation efficiency and biomass yields. To date, these strategies have resulted into up to two fold increases in biomass productivity in algae and crop yields in outdoor field trials.

Friday, August 5th, 2016, 9:30 AM - 10:00 AM
Systems Biology of Tension Wood Formation
Andrew Groover, USDA Forest Service, University of California, Davis
agroover@fs.fed.us
Suzanne Gerttula, US Forest Service; Matt Zinkgraf, US Forest Service; Heloise Bastiaanse, US Forest Service; Shawn Mansfield, University of British Columbia; Isabelle Henry, University of California, Davis; Luca Comai, University of California, Davis; Vladimir Filkov, University of California, Davis

Wood development is highly plastic, and is modified in response to several environmental cues. Leaning stems of angiosperm trees often produce specialize cells, called tension wood, in response to gravitational stimulus. Tension wood is characterized by increased cell division, altered growth, decreased numbers of vessel elements, and the production of fibers that contain a distinct cellulose-rich gelatinous cell wall layer that generates contractile force that allows the stem to resume upward growth.

In this presentation we describe two approaches for understanding tension wood formation in Populus. In the first approach, we use a gene co-expression network as a framework for integrating multiple genomics, phenotypic, and imaging data. We will describe co-expression gene modules that are highly correlated with tension wood developmental phenotypes, and show that the transcription factor ARK2 and the hormone gibberellic acid modulate the development of cell walls in these specialized wood fibers. In the second approach, we have established a large population of hybrid poplars carrying chromosomal lesions. These lesions have been precisely mapped using whole genome sequencing for each genotype, and create defined gene dosage variants that display distinct phenotypes. Results correlating dosage lesions to phenotypes, as well as specific mutants affecting tension wood development will be presented.

Friday, August 5th, 2016, 10:00 AM - 10:30 AM
Enhancing the Efficiency of Photosynthesis in C3 Plants
Maureen Hanson, Cornell University
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Myat Lyn, Cornell University; Alessandro Occhialini, Rothamsted Research; John Andralojc, Rothamsted Research; Martin Parry, University of Lancaster

Photosynthetic efficiency of C3 plants suffers from the slow catalytic rate and reaction of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) with O2 instead of CO2, leading to the costly process of photorespiration. Cyanobacteria and other photosynthetic prokaryotes increase the concentration of CO2 around Rubisco within microcompartments named carboxysomes for more efficient incorporation of inorganic carbon. Because of the carboxysome, cyanobacteria are able to use RuBisCO enzymes that possess the highest known catalytic rates even though such enzymes are more sensitive to oxygen than those in land plants A report in which the potential improvement in photosynthesis in C3 plants by incorporation of of the cyanobacterial carbon concentrating mechanism (CCM) into chloroplasts predicted that biomass could increase as much as 60%. We have explored the possibility of producing β-carboxysomes containing the faster cyanobacterial Rubisco enzyme from Synechococcus elongatus PCC7942. Using the agroinfiltration technique, we transiently expressed multiple β-carboxysomal proteins (CcmK2, CcmM, CcmL, CcmO and CcmN) in Nicotiana benthamiana with fusions that target these proteins into chloroplasts and that provide fluorescent labels for visualizing the resultant structures. By confocal and electron microscopic analysis, we have observed that the shell proteins of the β-carboxysome are able to assemble in plant chloroplasts into highly organized structures resembling empty microcompartments. Using chloroplast transformation, we have shown that the tobacco Rubisco enzyme can be replaced with a kinetically faster cyanobacterial enzyme, which assembles and confers autotrophic growth. After increasing the expression of cyanobacterial RuBisCO by improving the gene regulatory elements in our transgene locus, we obtained transformants whose growth approximates that of wild-type plants in a high CO2 environment. Our experiments establish the feasibility of introducing carboxysomes into chloroplasts, once additional cyanobacterial genes are incorporated under the control of appropriate regulatory elements.

Friday, August 5th, 2016, 11:00 AM - 11:30 AM
Algal Cultivation Strategies for Advanced Biomass Yield
Kim Ogden, University of Arizona
ogden@email.arizona.edu

This presentation will highlight research results that are striving to overcome critical barriers of cost, water resource management, and nutrient recycle to obtain long-term sustained domestic algal biomass production. A variety of sophisticated, monitored, and controlled cultivation systems for large-scale algal production in testbeds will be highlighted that include both new photobioreactors and open pond systems. We have developed a crop rotation strategy for cultivating algae in open systems year round. The algal strains consist of Chlorella sorokinia in the summer months, Scenedesmus obliquus in the fall and Monophoridum sp. in winter months. Our strategy is to 1) determine growth rate and productivity in the laboratory to evaluate the best strains for seasonal growth; 2) optimize media to reduce cost; 3) transfer information and stains to testbeds for outside growth; 4) cultivate strains semi-continuously monitoring pH, T, water, nutrients, DO, EC and OD continuously; and 5) develop new molecular techniques and sensors to monitor cultivation. Crop protection strategies for minimizing contaminant species will also be discussed.

Friday, August 5th, 2016, 11:30 AM - 12:00 PM
Modeling Carbon Flux in Photosynthesis
Jamey Young, Vanderbilt
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Engineering host metabolic phenotypes that promote high yield and specific productivity is a major goal of the biotech industry. 13C metabolic flux analysis (MFA) provides a rigorous approach to quantify host

metabolic phenotypes by applying isotope tracers to map the flow of carbon through intracellular metabolic pathways. In particular, transient measurements of isotope incorporation following a step change from unlabeled to labeled CO2 can be used to estimate photoautotrophic fluxes by applying isotopically nonstationary MFA (INST-MFA). We have recently developed a package of MATLAB routines called INCA that automates the computational workflow of INST-MFA. INCA is the first publically available software package that can perform INST-MFA on metabolic networks of arbitrary size and complexity. We subsequently applied dynamic 13C labeling experiments and INST-MFA to map the photoautotrophic metabolism of cyanobacteria that have been engineered to produce isobutyraldehyde (IBA). The flux analysis identified an alternative three-step route from PEP to pyruvate, which supplied the majority of carbon for IBA synthesis. Based on these results, we overexpressed each single enzyme involved in this pathway and identified a strain with significant improvement in IBA production.
We next adapted our INST-MFA model to a terrestrial plant system. We performed in vivo isotopic labeling of Arabidopsis thaliana leaves with 13CO2, measured the transient labeling of 37 metabolite fragment ions using mass spectrometry, and estimated fluxes throughout leaf photosynthetic metabolism using INCA. Leaves were acclimated to either 200 (LL) or 500 (HL) µmol/m2/slight intensity. Approximately 1,400 independent mass isotopomer measurements were regressed to estimate 136 fluxes under each condition. Despite a doubling in the carboxylation rate, the photorespiratory flux increased from 17% to 28% of net CO2 assimilation with HL acclimation. Interestingly, the concentrations of multiple Calvin cycle intermediates were reduced during acclimation, indicating an inverse relationship between intermediate pool sizes and fluxes. These studies have established 13C INST-MFA and the INCA software package as a comprehensive platform to map carbon fluxes in cyanobacteria, plants, and other photoautotrophic organisms.

Friday, August 5th, 2016, 12:00 PM - 12:30 PM

Transcriptome response to high CO2 concentrations of an environmental microalgae after eight years of enrichment: implications in carbon capture and byproduct generation

Adriana Pacheco Moscoa, Tecnológico de Monterrey
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C. Senes-Guerrero, Tecnológico de Monterrey

Biological fixation of CO2 by microalgae is a promising strategy to mitigate global warming. We collected and enriched a freshwater sample in air, 25, and 50% CO2 atmospheres, which resulted today in cultures with eight years of continuous CO2 exposure. After 1.5 years, only one microalgae strain survived and was phylogenetically characterized as Desmodesmus abundans strain RSM (UTEX No. 2976). At high CO2, strain RSM showed changes in morphology, tolerated and captured all gaseous CO2, generated higher biomass, and was able to grow with other flue gases components (NOx and SOx). Therefore, understanding global changes in gene expression may elucidate an adaptation strategy to high CO2 and help elucidate byproducts generated under this growth condition. We used RNA ¬sequencing to explore differentially expressed genes (DEGs) of RSM cultures exposed to high CO2, using the air culture as a control. Surprisingly, at 25% CO2 changes in gene expression were minimal (299 DEGs) while the opposite was observed at 50% CO2 (26,770 DEGs). Gene Ontology (GO) terms resulted in 4,628 and 106 annotated DEGs for 50% and 25% CO2, respectively. At 25% CO2, majority of DEGs were localized in the cytosol and cell wall for protein folding and ATP binding, whereas at 50% they mostly occurred in plastids for biosynthetic processes and ion binding. In general, energy, nucleotide, and carbohydrate metabolism were up regulated at 50% CO2. In contrast, no difference or down regulation of these pathways was observed at 25% CO2. It seems the 25% CO2 culture was scaling down from autotrophic growth as the sub-categories of photosynthesis and purine metabolism were down regulated, which corresponded to a low CO2 concentration in the gas phase (<1% CO2) at the moment of transcriptome

analysis. Results suggest that for this microalgae strain an atmosphere of 25% CO2 does not trigger many changes in their metabolic response to high CO2, while strong changes in gene expression are necessary at 50% CO2 where the culture is active in energy and lipid metabolism.

Friday, August 5th, 2016, 1:30 PM - 2:00 PM
Computational studies of components of plant cell wall and thylakoid membrane
Sandrasegaram Gnanakaran, LANL
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Challenges encountered during the conversion of biomass to fuels are critically linked to the physical properties of the feedstock. The first part of the talk will address the utilization of a multitude of computational techniques, including agent based model, statistical mechanical and coarse-grained models, all-atom molecular dynamics simulations and quantum chemical calculations towards a wide range of biomass conversion problems. Also, we will discuss the stochastic models developed at LANL to capture spatiotemporal aspects of catalytic degradation of carbohydrates. The second part of the talk will address the challenge of photoinhibition and its amelioration by Non Photochemical Quenching (NPQ). A molecular level understanding of organization of protein complexes along with chlorophylls and carotenoids in the thylakoid membrane of plants (and algae) is critical for understanding energy capture and photoprotective processes. Among the photoprotective mechanisms, NPQ is the most effective mechanisms to reduce photodamage in plants. We have modeled the first all-atom model of the thylakoid membrane. Recently, using this model of thylakoid membrane, we have computationally engineered a rapidly responding, pH-sensitive protein switch that alters the protein conformation of the light harvesting antenna protein, CP29, accelerating the dissipation of excess energy through NPQ process.

Friday, August 5th, 2016, 2:00 PM - 2:30 PM
Elucidating the Mechanism of Xylan Biosynthesis: From Genes to Function
Breeanna Urbanowicz, University of Georgia
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Maria Peña, University of Georgia; Jeong Yeh Yang, University of Georgia; Abigail Agyeman, University of Georgia; Kelley Moremen, University of Georgia; William York, University of Georgia

The production of plant biopolymers and functional metabolites requires the coordinated action of numerous enzymes. The hemicellulose 4-O-methyl glucuronoxylan is one of the principle components present in the secondary cell walls of eudicotyledonous plants. However, the biochemical mechanisms leading to the formation of this polysaccharide and the effects of modulating its structure on the physical properties of the cell wall are poorly understood. In order to alter or assemble new metabolic pathways, we must first gain a better understanding of these intricate enzymatic systems, which has proven difficult since obtaining plant proteins in pure, active form has been met with limited success. Recently, we have optimized construct design and heterologously expressed over 40 carbohydrate active enzymes that participate in the biosynthesis of plant cell wall polysaccharides in mammalian cells demonstrating that this is a robust heterologous expression system for these Golgi-resident enzymes. This unprecedented access to large amounts of pure enzymes opens up a new doorway to perform structure-function studies and to investigate complex biochemical pathways in vitro. This knowledge provides new opportunities to selectively manipulate structure and extends the portfolio of targets that can be modified either alone or in combination to modulate biopolymer interactions in the plant cell wall.

Friday, August 5th, 2016, 2:30 PM - 3:00 PM
New insights into the structure and function of plant cellulose synthase and the cellulose synthesis complex
Candance Haigler, North Carolina State University
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Due to its abundance within plant cell walls, cellulose is a main source of glucose within biomass crops for conversion into biofuels. The potential for rational engineering of cellulose synthesis in plants has emerged within the last three years due to new structural insights. Through computational modeling, we now have a better understanding of the structure of the plant cellulose synthase (CESA), although no plant CESA structure has so far been solved empirically. To resolve a potential discrepancy as compared to prokaryotic cellulose synthase, biochemical experiments have been conducted to determine the membrane topology of CESA. Engineered CESAs have been used in phenotype complementation assays in CESA mutants to demonstrate the functional importance of key regions of the protein, while at the same time revealing differences between isoforms. Efforts to refine and complete the CESA predicted structure will be described. Analysis of the stability of computational assemblies of multiple CESA models and spatial comparison of models to improved images of the multi-protein cellulose synthesis complex (CSC) have provided evidence for a maximum of 18 CESAs within one CSC. This in turn supports the likelihood of 18 glucan chains within one fundamental cellulose microfibril, although two or more of these may assemble into larger fibrils within the plant cell wall. Overall, more definitive results are rapidly emerging to allow strategies to be developed and implemented for beneficial manipulation of cellulose synthesis in plants. This work reflects the efforts of a multidisciplinary group supported as part of The Center for LignoCellulose Structure and Formation, an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Basic Energy Sciences under Award # DE-SC0001090.

Friday, August 5th, 2016, 3:30 PM - 4:00 PM
Modeling Molecular Structure of a Cellulose Synthase Plant Conserved Region with X-ray Crystallography and Small Angle X-ray Scattering Illustrates Potential Functions
Phillip Rushton, Purdue University
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Anna Olek, Purdue University; Lee Makowski, Northeastern University; John Badger, DeltaG Technologies; C. Nicklaus Steussy, Purdue University; Cynthia Stauffacher, Purdue University; Nicholas Carpita, Purdue University

The processive plant cellulose synthase (CesA) synthesizes (1→4)-β-D-glucans. Several dozen CesAs assemble into a complex that forms a cellulose microfibril as the fundamental scaffolding unit of the plant cell wall. Within the CesA catalytic domain (CatD) is a 125-amino acid insertion known as the plantconserved region (P-CR), whose function and molecular structure are unknown. Recombinantly expressed rice secondary cell wall OsCesA8 P-CR domain purifies as a monomer and shows distinct α-helical secondary structure by circular dichroism analysis. A molecular envelope of the P-CR was derived by small angle X-ray scattering (SAXS). The P-CR was crystallized and structure solved to 2.4Å resolution revealing an anti-parallel coiled-coiled domain that has an uncommonly high proportion of aromatic residues intercalated in 'knob-in-hole' heptad positions. Connecting the coiled-coil α-helices is an ordered loop that bends back towards the coiled-coils and forms several hydrophobic interactions. The P-CR crystal structure fits the molecular envelope derived by SAXS, which in turn fits into the CatD molecular envelope as predicted in previous work (Olek et al., 2014, Plant Cell 26:2996). This places the P-CR between the membrane and substrate entry portal with the connecting loop facing the catalytic core, where it is likely making a hydrophobic contact. This positioning indicates that the P-CR could

function in protein-protein interactions in the cellulose synthase complex (CSC) through its coiled-coil and/or substrate entry through its N- and or C-terminus. Understanding the molecular structure of CesAs, CesA protein-protein interactions and substrate/product pathways is critical to designing novel CesA mutants and chimeras that produce modified cellulose microfibrils for improved catalytic conversion of biomass to biofuels.

Supported by the Center for Direct Catalytic Conversion of Biomass to Biofuels (C3Bio), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Award Number DE-SC0000997.

Friday, August 5th, 2016, 4:00 PM - 4:30 PM

Cell Wall Metabolism and Biomass of Sugarcane May Change Under Different Stressing Conditions Related to the Global Climate Change

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De Souza, University of São Paulo, University of Illinois; A.P. Piovezzani, University of São Paulo; A.R & Buckeridge, University of São Paulo

In Brazil, sugarcane genetics promoted development of varieties that are highly productive and this has led Brazil to become the second larger producer of ethanol in the world, the 1G being the main process used and 2G starting to become commercially available. Indeed, there are estimates that Brazilian sugarcane (1G+2G), if used sustainably without affecting food production or forests, could offset more than 10% of the CO2 emissions even considering the estimated reduction in water availability. However, in order to realize this potential, it is important to understand whether biomass composition, and consequently, the production of 2G ethanol, will be affected by the Global Climate Change (GCC). In this work we report how biomass production and the expression cell wall-related genes in the whole plant of sugarcane grown under elevated CO2, drought and the combination of both. Biomass production increased under elevated CO2 and the negative response (biomass decrease) induced by drought was offset by CO2 elevation. The levels of expression of synthases and hydrolases were higher in culm (stem) than in leaves. Expressions of biosynthesis- and hydrolysis-related genes were found in leaves and stems, the two organs used for 2G bioethanol production. In both cases distinct sugarcane expression clusters (SAS – putative gens) related to different cell wall domains (cellulose, hemicellulose, pectin, lignin) were found under the stressing conditions used (elevated CO2, drought and the combination of both). These results suggest that the control of cell wall metabolism in the growing plant will depend on environmental changes. This approach also afforded a systemic view of cell wall metabolism throughout the whole plant, what will probably allow prediction of subtle changes in biomass composition under those stresses. The discovery that genes related to several classes of endogenous hydrolases and synthases are active within stem and leaves of sugarcane points out to candidate genes to be used in biological pretreatment of biomass for bioenergy production. We conclude that cell walls may change due to the stresses related to the Global Climate Changes in the future.

Financed by CNPq and FAPESP under the National Institute of Science and Technology of Bioethanol and the Microsoft-FAPESP Institute.

Friday, August 5th, 2016, 4:30 PM - 5:00 PM

Mesoscale modeling of coupled transport phenomena and conversion kinetics in plant cell walls and biomass particles

Peter Ciesielski, NREL

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Michael Crowley, National Renewable Energy Lab; Byron Donohoe, National Renewable Energy Lab; Michael Himmel, National Renewable Energy Lab

The overall rates and yields biomass of conversion are determined by several complex physiochemical processes that occur in tandem over multiple length and time scales. First, chemo- and bio-catalysts (and heat in the case of thermochemical conversion) must enter biomass particles via through various routes within the tissue structure. Next, these catalysts must penetrate the cell wall through a complex network of biopolymers. Once inside (or in close proximity to) the cell wall, chemical reactions occur between catalysts and biopolymers that convert the macromolecules into smaller, soluble or volatile products. Finally, the products of these reactions must exit the remains of the cell wall matrix and the particle. In real biomass conversion scenarios, the simultaneous occurrence of these processes give rise to the rates and yields observed at the bulk scale. Improving our understanding of each of these interdependent, dynamic processes at a fundamental level will provide insight that can be used for optimization of the processes in their entirety. Furthermore, developing coupled, multiscale simulations for these processes will allow for high-throughput investigations of various conversion scenarios in silico, and provide some predictive utility regarding the efficacy of new processes. In this presentation, I will describe our efforts toward multiscale simulations of biomass conversion. I will focus on recent progress with mesoscale simulations that incorporate biopolymer structure at the cell wall scale and biomass tissue structure at the particle scale. Constructive solid geometry (CSG) operations are used to represent the complex organization of cellulose fibrils, lignin, and hemicellulose at the cell wall scale; and cellular geometry at the tissue/particle scale. The resultant structures are imported in finite element analysis software are used in simulations of transport phenomena coupled to various conversion kinetic schemes. I will conclude by presenting several applications of these methods wherein thermochemical and biochemical conversion processes are simulated.

Friday, August 5th, 2016, 5:00 PM - 5:30 PM

Feruloyl transferases AT5, AT9 and AT10 in the BAHD family are involved in cell wall biosynthesis in grasses

Alex Yi-Lin Tsai, Joint BioEnergy Institute
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Herik Scheller, Joint BioEnergy Institute; George Wang, Joint BioEnergy Institute; Edward E. K. Baidoo, Joint BioEnergy Institute; Aymerick Eudes, Joint BioEnergy Institute; Chengcheng Zhang, Joint BioEnergy Institute; Dominique Loqué, Joint BioEnergy Institute; Jay Keasling, Joint BioEnergy Institute; Laura Bartley, Joint BioEnergy Institute

BAHD is a plant-specific acyltransferase superfamily involved in synthesis and modification of plant secondary metabolites. A subclade of BAHD proteins, designated here as the “Mitchell clade”, is highly expanded in grasses and has been proposed to be involved in synthesis of hydroxycinnamate esters in the grass cell wall (Mitchell et al., 2007). The putative CoA-acyltransferases (ATs) in the Mitchell clade have not been well characterized to date, as only one (AT4/PMT) of the twenty members in rice (Oryza sativa) has been biochemically characterized as a pcoumarate monolignol transferase (Withers et al., 2012). Previous work examining rice plants with elevated expression of AT5 and AT10 revealed altered hydroxycinnamic acid content in the cell wall, consistent with BAHD members playing a role in cell wall biosynthesis (Bartley et al., 2013). In this study, we have biochemically characterized the members of rice BAHD acyltransferases. We have attempted to express AT5 and AT10 in conventional expression hosts with no avail. Herein, we used a yeast in vivo assay to screen for the activities of rice BAHD acyltransferase from the Mitchell clade. Saccharomyces cerevisiae constitutively co-expressing

Arabidopsis 4CL5 (4-coumarate:CoA ligase 5) and rice AT5 was simultaneously fed various combinations of hydroxycinnamic acids and monolignols as acyl donors and acceptors, respectively. This resulted in the formation of coumaryl ferulate and coniferyl ferulate when the respective donor-acceptor pairs were provided to the yeast culture. The absence of product formation with p-coumaric acid as donor suggests that AT5 is a relatively specific feruloyl-CoA acyltransferase. The absence of product when feeding the same substrate pairing to yeast expressing rice AT10 suggest it is functionally distinct from AT5. AT9 was expressed in yeast and purified. While AT5 appears to be involved in lignin biosynthesis, AT9 may be involved in adding hydroxycinnamates to xylan. The activity of AT9 will be reported.

Saturday, August 6th, 2016, 8:30 AM - 9:00 AM

Myosins XI are involved in trafficking of cellulose synthase complexes in Arabidopsis

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Cellulose microfibrils, the major tensile components of the plant cell wall, play essential roles in normal plant growth and development. Cellulose, as the most abundant biological polymer on earth, is also the raw material used for production of paper, textiles and biofuels. In higher plants, cellulose is synthesized at the plasma membrane (PM) by large cellulose synthase complexes (CSC) consisting of multiple cellulose synthase (CESA) proteins. Cellulose production is largely influenced by the rate of intracellular trafficking of CSCs and their lifetime at the PM. CSCs are believed to be assembled in the Golgi apparatus and delivered to the PM via small microtubule-associated transport vesicles, and their uptake into the cytosol is mediated by clathrin-based endocytic machinery. Both processes are likely to involve the actin cytoskeleton serving as tracks for vesicle or organelle transport, as well as clathrin-coated vesicle formation. Myosins XI are motor proteins that move diverse organelles and vesicles along actin filaments, thus playing a critical role in organelle trafficking in plant cells. However, the molecular mechanisms by which actin and myosin contribute to CSC trafficking or cellulose deposition remain obscure. In this study, measurement of cellulose content suggested that cellulose biosynthesis was greatly reduced in a myosin xik xi1 xi2 triple knockout (3KO) mutant. By quantitative image analysis of living epidermal cells expressing YFP-CESA6 in both the xi3KO mutant and myosin inhibitor-treated cells, we showed that myosin is involved in regulating both the delivery and uptake of CSCs at the PM. Moreover, altered cytoskeletal dynamics in actin capping protein (cp) mutants also perturbed CESA trafficking and PM dynamics. These are the first data implicating myosin in cellulose production in higher plants.

Saturday, August 6th, 2016, 9:00 AM - 9:30 AM

Impact of Climate Change on Forest Ecosystems

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Temperature has risen ~1 C° since 1880 and has already been attributed with global crop declines and widespread forest mortality, despite the benefits of CO2 fertilization. Temperature is predicted to rise and additional 1 to 5 C° by 2100. Rising temperature forces greater evaporation, causing even relatively mild droughts to have massive impacts on plant photosynthesis, yield, and survival. Maximizing our global carbon cycle, our natural resources, and our fuel and food supply under this rising stress will require significant changes in how we manage ecosystems. I will review the state of knowledge on this growing threat and on how we can adapt our agricultural systems to minimize carbon losses and maximize carbon gains.

Saturday, August 6th, 2016, 9:30 AM - 10:00 AM

Techno-economic and Life Cycle Assessment: Integration of Experimental Systems with Engineering Process Modeling

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Globally, we face an energy crisis due to an increase in energy consumption combined with the negative effects associated with traditional fossil energy sources. A variety of green technologies coming together to address environmental concerns, while meeting global increases in energy demand, is likely to be a critical component of the solution. This seminar presents the integration of experimental systems with engineering process modeling for sustainability assessment algal based biofuels systems. Sustainability modeling includes techno-economic assessments, life cycle assessments, and scalability assessment through resource availability. Data feedback from sustainability modeling is used to highlight areas for focused research and development on the metrics of economic viability and environmental impact. Further, engineering process modeling is used to identify knowledge gaps for experimental work. The integration of sustainability modeling with experimental systems is a valuable tool that can decrease experimental design space and focus research in areas that can accelerate commercialization. Experimental research spans the value chain of a microalgal biorefinery system with the focus on the generation of data for system model validation. Results for multiple growth architectures and downstream processing systems are presented.

Saturday, August 6th, 2016, 10:00 AM - 10:30 AM

The role of the Mediator complex in the regulation of lignin deposition

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The products of the phenylpropanoid pathway range from complex, insoluble polymers such as lignin and suberin, to soluble flavonoids and hydroxycinnamate esters, to volatile compounds used to attract pollinators. In addition to its important role in plant biology, lignin has a significant impact on the effectiveness of converting cell wall polysaccharides to ethanol or second-generation biofuels. For this reason, a great deal of research has focused on altering phenylpropanoid metabolism through mutation or RNAi-mediated down-regulation of genes encoding pathway enzymes. Although many of these manipulations lead to significant alterations in plant metabolism, defects at a number of biosynthetic steps lead to a common suite of pleiotropic phenotypes such as dwarfing and sterility, the severity of which is dependent on the strength of the metabolic restriction. Analysis of the reduced epidermal fluorescence4 (ref4) mutant has revealed that the REF4 protein play a role in the suppression of phenylpropanoid biosynthesis in wild-type plants. REF4 and its paralog, REF4-related 1 (RFR1), have recently been shown to be components of Mediator, a large multi-protein complex that facilitates interactions between DNA-bound transcription factors and RNA polymerase II. Mutants of Arabidopsis that lack REF4 and RFR1 hyperaccumulate phenylpropanoids and show little in the way of developmental changes. Surprisingly, ref4/rfr1 mutations mitigate the dwarf phenotype and sterility of the ref8 mutant of Arabidopsis which is defective in the early phenylpropanoid pathway enzyme p-coumaroyl shikimate 3-hydroxylase. Our data reveal that dwarfism in at least some phenylpropanoid pathway mutants is the result of a cascade of transcriptional mis-regulation that is dependent on Mediator.

Saturday, August 6th, 2016, 10:30 AM - 11:00 AM
Chemical Conversion of Carbohydrates to Alkanes
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According to a recent report, lignocellulose is the most abundant renewable biological resource on earth, with an annual production of ~ 200 x 109 tons.1 Conversion of lignocellulosics derived from wood, agricultural wastes, and woody grasses into liquid fuels and value-added chemical feedstocks is an active area of research that has seen an explosion of effort due to the need to replace petroleum based sources. The carbohydrates D-glucose (C6), L-arabinose (C5), and D-xylose (C5) are readily obtained from the hydrolysis of lignocellulose and constitute the most abundant renewable organic carbon source on the planet. Because they are naturally produced on such a large scale, these sugars have the greatest potential to displace petrochemical derived transportation fuel.2 Recent efforts in our laboratories aimed towards the production of high energy density transportation fuels from carbohydrates have been structured around the parameters of selective carbohydrate carbon chain extension chemistries, low reaction temperatures, and the desired use of water or neat substrate as the solvent. Some of our efforts in this regard will be presented.

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Saturday, August 6th, 2016, 11:00 AM - 11:30 AM
Regional Feedstock Partnership Data Underpin Yield Assumptions in the 2016 Billion Ton Update
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DOE's Bioenergy Technologies Office is focused on applied research, development and demonstration of technologies that produce fuels and chemicals from renewable biomass, including algae and higher plants. BETO may be best known to this group because of the Billion-Ton Study (2005) and its subsequent Updates in 2011, and again in July, 2016. The BT16, as we fondly refer to the most recent Update, is a further refinement of potential amounts of lignocellulosic feedstocks available on a county level basis at various farmgate costs over time. What many people do not realize is that the crop yields assumed in the original Billion-Ton Study were gathered from groups of experts at workshops convened by BETO (then the Office of the Biomass Program). Yield assumptions used in subsequent updates have relied heavily on field trials performed by a large multi-institutional consortium of university and USDA-ARS scientists over an eight year period in a project funded by BETO. This talk will discuss the results produced by the Regional Feedstock Partnership (RFP), and how their results have influenced assumptions in the BT16 report. RFP collaborators have also collected biomass samples from their plots each year and provided them to Idaho National Lab for characterization. These samples are playing a key role in the Feedstock-Conversion Interface Consortium, which BETO is in the process of standing up now. BETO hopes to broaden this Consortium beyond the national lab system in coming years.

Saturday, August 6th, 2016, 11:30 AM - 12:00 PM
The Green Marathon: Algal Cultivation, Crop Protection, Co-products and Conversion as NMSU
Omar Holguin, NMSU

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We describe the algal biofuel research activity and capacity at New Mexico State University through recent highlights that include algal strain development, crop protection research, the identification and development of co-products, remediation and use of alternative water resources and thermochemical biomass conversion. We describe both indoor and outdoor cultivation of NMSU's production strains and illustrate changes in biochemical composition with stress, and wastewater treatment with mixotrophic algal cultivation including pilot-scale demonstration. We demonstrate the utility of complex mixture analysis by advanced mass spectrometry to describe algal lipid biochemistry and provide comprehensive compositional description of complex bio-crude oils from thermochemical algal biomass treatment and the downstream processing of those materials. Finally, we summarize our observations of the hydrothermal liquefaction of different microalgal feedstocks at variable processing conditions.

Saturday, August 6th, 2016, 12:00 PM - 12:30 PM
RNAi suppression of lignin biosynthetic genes in sugarcane improves glucose release without impacting sucrose production
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Sugarcane is a sub-tropical crop that produces large amounts of biomass annually. It is a key agricultural crop in many countries for the production of sugar and other products. Currently the bagasse remaining following sucrose extraction is underutilized in many cases and this bagasse has great potential as a carbohydrate source for the production of biofuels. As with all lignocellulosic crops, lignin provides a barrier of access for carbohydrates, and as such, is a focus of transgenic efforts. In this study we used RNAi to reduce expression of three key genes in the lignin biosynthesis pathway individually in sugarcane. These genes, caffeoyl-CoA Omethyltransferase (CCoAOMT), ferulate-5-hydroxylase (F5H) and caffeic acid Omethyltransferase (COMT), have all been shown to impact lignin content or composition in other species. For each construct, three events were selected for further analysis based on qPCR results. For the CCoAOMT lines, there were no lines with a reduction in lignin content and no lines showed improved glucose release. For F5H, no lines had reduced lignin, but one line had a significant increase in glucose release. For COMT, one line had reduced lignin content and also had higher levels of glucose release by enzymatic hydrolysis. The two lines with improved glucose release (F5H-2 and COMT-2) also had reduced S:G ratios. Despite improvement in bagasse qualities for the production of lignocellulosic based fuels, there was no reduction in juice sucrose extraction, providing evidence that the alteration of sugarcane for improved lignocellulosic ethanol production can be achieved without negatively impacting sugar production.

Sunday, August 7th, 2016, 9:00AM - 9:30AM
Intergrating Biology and Chemistry for the Production of Bioproducts
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The replacement of fossil carbon-derived chemicals and fuels with bioproducts is both a technical and economic challenge. From a technical perspective the challenge is how to selectively and efficiently remove oxygen from highly oxygenated substrates. However, the economic challenge is possibly even

more significant in that bioproducts must compete against a highly efficient fossil carbon-based production system. To successfully compete bioproducts must be produced through the most effective coupling of biology and chemistry. However, these technical communities are challenged to find a clear contextual basis for advancing this coupling in a systematic manner. One such approach is to develop a technological framework in which a range of bioproducts can be produced from a common platform. A generalized platform being developed by the NSF Engineering Research Center for Biorenewable Chemicals (CBiRC), depends on the exploitation of the fatty acid/polyketide metabolic pathway leading to a diversity of intermediate chemicals that are subsequently converted to bioproducts products using heterogeneous catalysts. An overview of the technical strategy being used by CBiRC to achieve systematic integration of biology and chemistry as well as insights learned from this integration will be discussed.

Sunday, August 7th, 2016, 9:30 AM - 10:00 AM

Rice AP2/ERF transcription factor overexpression increases biomass accumulation and grain yield while simultaneously enhancing tolerance to abiotic stress

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Plant lignocellulosic material is currently being used to generate biofuels. To make this system more efficient by increasing plant biomass, a specific T-DNA expression cassette was engineered to increase phloem loading of sucrose via vascular expression of a gene involving hyperactive sucrose transport within rice (Oryza sativa). By screening numerous transgenic plants generated from this specific TDNA insertion we discovered a single plant that was noticeably larger than its counterparts. This plant's T-DNA insertion was found to be truncated, only containing the selective marker (hygromicin-resistance gene) and a portion of a companion cell specific promoter. The presence of the insertion was tracked over multiple generations and directly correlated with increases in all of the biomass characteristics measured. The mutant plants had a 7.4-fold increase in biomass and a simultaneous 3.6-fold increase in seed yield compared to segregating wild-type plants in the initial screen. Given the substantial increase in biomass shown by the mutant we refer to it as mpg1 (makes plants gigantic-1). We hypothesize that the insertion caused a mutagenic event that resulted in altered expression of a nearby gene(s). RT-PCR, along with more comprehensive phenotyping, has led to the discovery of a candidate transcription factor (TF) that is over-expressed when the partial T-DNA construct is present. Moreover, the mpg1 mutant's growth is resistant to stressful growth conditions that decrease wild-type plant yields. Identification of the mechanism responsible for the increased biomass in mpg1 may lead to strategies that could be applied to bioenergy and/or food-relevant crops, such as Miscanthus, switchgrass, sorghum, rice, and corn.

Sunday, August 7th, 2016, 10:00 AM - 10:30 AM

Phototropin; A Master Regulatory Control Gene that Controls Photosynthesis and Growth Processes in Chlamydomonas

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Phototropin is a family of blue light receptors, which mediate a variety of physiological processes in plants. In higher plants these processes include; phototropism, chloroplast movement and stomatal opening. In contrast to plants, however, the green alga Chlamydomonas reinhardtii has only a single phototropin (PHOT) gene. In Chlamydomonas PHOT has previously been shown to play a central role in controlling sexual life cycle, eye spot size, and light sensitivity. PHOT has also regulates chlorophyll, carotenoid, and light-harvesting chlorophyll binding protein abundance in algae. Recently, we compared the growth and photosynthetic rates of Chlamydomonas phototropin knock-out (PHOT KO) mutants to their parental wild-types. We observed 2-fold higher growth rates associated with substantial increases in CO2-dependent rates of photosynthesis in PHOT KO mutants. Comparative analyses of the transcriptomes of a PHOT KO mutant to its parental wild-type (WT) demonstrated significant increases in transcript levels for genes involved in photosynthetic electron transport, carbon fixation, starch and lipid synthesis, and those regulating cell cycle and cell division control. With respect to facilitating photosynthetic electron transfer, we observed elevated transcript levels for genes encoding proteins of the cytochrome b6f and ATP synthase complex, as well as increased lumenal volumes in PHOT KO mutants potentially facilitating protoncoupled electron transfer. In addition, transcripts encoding proteins involved in limiting steps in the Calvin cycle including; RuBisCO, SBPase, and 3PGDH were more abundant in PHOT KO mutants than WT. Consistent with enhanced photosynthetic traits, genes encoding proteins involved in regulating sink strength and cell division including; starch synthase, fatty acid biosynthesis, known master regulatory genes regulating plant growth, and cell-cycle control genes (CDK) were also substantially up-regulated in PHOT KO mutants. In conclusion, our studies suggest that phototropin may be a master regulatory gene that suppresses rapid cell growth and promotes gametogenesis and sexual recombination in wild-type strains but allows for rapid growth when inactivated in PHOT mutants. (Supported by grants from the Department of Energy).

Poster Abstracts

P1 *The Development of Setaria as a Model System to Investigate Key Bioenergy Feedstock Traits*
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Andrew Eamens, University of Newcastle; Robert Furbank, Australian National University; John Lunn, Max Planck Institute of Molecular Plant Physiology; Joseph Pegler, University of Newcastle; Kate Chamberlain, University of Newcastle; Duc Quan Nguyen, University of Newcastle; Christopher Brown, University of Newcastle; William Palmer, University of Newcastle; Antony Martin, University of Newcastle

Although Setaria has been proposed as a model to investigate C4 photosynthesis, it may also be considered a suitable representative for biofuel feedstocks which are predominantly closely related panicoid grasses. In order to extend our understanding of the fundamental molecular and physiological mechanisms underpinning both sugar storage and cell wall deposition as they occur during plant development, we have investigated an elongating stem internode of S. viridis. The chosen internode progressed from an active meristem and primary cell wall region at the base of the internode towards maturing fully expanded cells at the top of the internode. Along this developmental gradient, RNAseq of both the mRNA and sRNA fractions of the transcriptome was undertaken. These two RNA-Seq datasets are to be mapped to the S. viridis genome to establish a global transcriptome gene expression atlas along the developmental zones of the elongating internode. Further, we will use our recently established S. viridis transformation system for manipulation of candidate genes identified in our ‘omics’ approaches.

A holistic understanding of the synthesis, composition and structure of the cell wall and the molecular mechanisms that signal the transition from primary to secondary cell wall synthesis will be integral to engineering crops with higher ratios of fermentable sugars and a structure that lends itself to more efficient deconstruction.

P2 *Exploring novel photosynthetic functions during the acclimation of Chlamidomonas reinhardtii to nitrogen deprivation*

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The availability of macro-elements is a major factor dictating the growth rate and productivity of photosynthetic organisms. Consequently, substantial efforts (scientific and economic) are made in modern agriculture to enrich the soil with the necessary nutrients. Yet in nature, photosynthetic bacteria, algae and plants are frequently exposed to limited availability for those nutrients; forcing them to activate a series of acclimation responses. As a part of these responses, the photosynthetic apparatus can be dramatically modified in order to meet with the energetic and metabolic constrains caused by the deprived environment. Nitrogen (N) is a fundamental element for many cellular processes and it is an essential component in amino and nucleic acids. Importantly, N is also a main element in the photosynthetic apparatus and can be found in chlorophyll and in the abundant poly peptides - Rubisco and LHCs. During N deprivation, the capacity to de-novo synthesize amino acids is reduced and therefore the imbalance between N and carbon feedback on Calvin-BensonBasham cycle to inhibit (or slow down) carbon fixation. As a result, the major sink for electrons and other photo-products is not fully available. In Chlamydomonas reinhardtii, this constrain triggers a set of reaction within the photosynthesis machinery in order to avoid damaging by the light energy. We found, for the first time, that several energy and electrons flow pathways are integrated in order to reduce the excitation pressure on PSII, and that the type-II NADPH dehydrogenase (NDA2) is a main key player that administrates these interactions. NDA2 dependent cyclic electron flow around PSI is up-regulated, allowing the oxidation of NADPH and the reducing of the PQ-pool. Electrons in the PQ-pool can be either respired through chlororespiration by PTOX2 or be transferred to cyt b6f. The latter will cause an increase of the trans-membrane ΔpH and qE will be activated. Deep understanding of the acclimation responses of the photosynthetic machinery during N deprivation allow as exploring novel functional units that may affect or regulate the dynamic of the apparatus. By applying an ecophysiologycal screen on collection of “GreenCut” mutants’ strains we are able to detect new components in the acclimation response of Chlamydomonas reinhardtii to N derivation.

P3 *Regulation of germination and growth of bioenergy crops by nanotechnological approach*

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Mohamed Hassen, University of Arkansas at Little Rock; Kamal Pandey; University of Arkansas at Little Rock

Bioenergy crops are attractive plants that are used for energy production. Good plant candidate for bioenergy crop should produce high amount of biomass. Previously, we demonstrated the possibility to use a nanotechnological approach to enhance the productivity of model plant species (Khodakovskaya et al., 2011) as well as valuable food crops (Lahiani et al., 2013). The introduction of Carbon-based Nanomaterials (CBN) can activate seed germination and increase the growth of exposed plants. Here,

we tested the impact of two CBN on germination and biomass production of two important bioenergy crops: sorghum and switchgrass. Application of graphene and carbon nanotubes increased germination rate of switchgrass seeds and led to early germination of sorghum seeds. Expression analysis revealed that both tested carbon-based nanomaterials stimulated expression of aquaporin (water channel gene) in treated switchgrass plants. Thus, our results indicated the high potential of CBN to induce biomass production in bioenergy crops.

P4 *High-level, inducible expression of cellulases in poplar*
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Ethanol produced from lignocellulosic biomass is considered to be one of the potential replacements for fossil fuels. Economically competitive cellulosic ethanol production requires low-cost production of large amounts of cellulases. Currently, microbe-derived cellulases are too expensive for affordable cellulosic ethanol production. One of the most promising cellulase platforms is the expression of cellulases in planta, especially within the feedstock itself. In the study, we apply an inducible hyperexpression platform, In Plant ACTivation (INPACT) to highly express cellulases after ethanol induction in poplar. INPACT technology utilizes an ethanol inducible promoter to prevent negative pleiotropic effects on plants, and Gemini virus replication machinery to achieve high expression of recombinant proteins. We will evaluate the INPACT technology in poplar using β- glucuronidase (GUS) as the reporter protein. Following confirmation of the INPACT system, we will use INPACT to express thermotolerant cellulases in poplar. Selected cellulases from thermophilic organisms have been plant codon optimized and synthesized. A transgenic poplar line with high-level inducible expression of a replication initiation protein, required to activate the INPACT system, has been selected as a 'mother line'. This line has been supertransformed with constructs for the production of GUS and these lines are currently being evaluated. Production of cellulases within the biofuels feedstock, in this case poplar, holds great potential for reducing costs associated with lignocellulosic fuel production.

P5 *Genetic variation between three strains of Chlorella sorokiniana and high throughput screening of potentially beneficial commensal bacteria*
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Shawn Starkenburg, Los Alamos National Laboratory

The freshwater chlorophyte genus Chlorella is an algal production strain of high interest in biotechnology applications. Here we present the genome sequences and gene annotations of three strains of Chlorella sorokiniana and present the results of a comparative analysis of gene content and genome structure between these strains (DOE 1412, UTEX 1228 and 1230). Genome sequencing and assembly was performed using a combination of Illumina and Pacific Biosciences platforms. Though classified as the same species, we report a significant disparity of gene content, with each of the strains containing a large complement of strain specific genes (~40-200 genes per strain). Analysis of these unique genes, as well as genes shared between two of the three strains, may provide evidence to distinguish evolutionary history of these organisms and why adequate growth conditions vary between strains. Large numbers of genome rearrangements are also seen between the three strains and genome size varies by up to 3 Mb. In addition to genomics work, we present a high throughput, novel micro-scale method for co-culturing bacterial and algal species. This method is applied to C. sorokiniana DOE 1412 and amenable to other algal species in a number of potential applications including identification of bacterial cohorts beneficial to enhance growth rate and production of high value products.

P6 *Engineering of Camelina Sativa with self-adjustingLight-Harvesting antenna size*
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Natalia Friedland, New Mexico Consortium; Richard Sayre, New Mexico Consortium

Imbalance of light utilization between different levels of plant canopy is a major bottleneck in improving net photosynthesis in monocultures and therefore crop yields. Here we use an approach of reducing the optical crosssection of photosynthetic light-harvesting antenna size for equalizing the amount of light absorption through all levels of plant canopies. Since chlorophyll b is present only in the peripheral light harvesting antenna complexes, changing chl b levels leads to alterations in antenna size. Camelina Sativa with optimized chl b levels (chl a/b ratio 4 vs native chl a/b ratio 3) yield 40% more biomass in the field than wild-type (N. Friedland et al, unpublished data). Further improvements in light capture and energy conversion have been achieved in algae by engineering selfadjusting light-harvesting antenna that respond to altered light intensities (S.Negi at al, unpublished data). This was accomplished by using the light-regulated translational inhibitor NAB1 (Nucleic acid binding protein 1) which under high light conditions binds to specific mRNA site (Light Responsive Element, LRE) and inhibits protein synthesis. LRE was fused to 5' end of CAO gene which catalyzes the synthesis of Chl b. Transgenic algae were able to adjust antenna size depending on level of light intensity they were grown under. We have introduced the same approach into crop plants. A CAO-lacking Arabidopsis mutant (Chlorina) was used to engineer transgenic plants with LRE fused to the 5' end of the CAO gene and the NAB1 gene was expressed under the control of the light-sensitive cab-1 or psaF promoters. Transgenic plants having different Chl a/b ratios when grown under low versus high light were generated suggesting involvement of the NAB1 system in regulation of CAO synthesis. Plant phenotypes are currently being assessed.

P7 *Developing Opuntia ficus-indica as a low-input biofuels crop and a model for cuticular wax synthesis*
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Won Cheol Yim, University of Nevada; Rebecca Albion, University of Nevada; David Shintani, University of Nevada; Dylan Kosma, University of Nevada; John Cushman, University of Nevada

Increased duration and intensity of drought associated with global climate change will require drought-tolerant crop production systems. Increased biofuels production on semi-arid lands will demand crops that possess reduced water and nutrient requirements. High productivity and water-use efficiency make prickly pear cactus (Opuntia ficus-indica) a promising feedstockfor bioethanol, biogas, and other biofuels. In addition, the thick epicuticular wax layer of Opuntia makes it an ideal system for studying wax synthesis as it pertains to drought tolerance. The overall goals of this research program are to 1) reengineer Opuntia to produce and store lipids instead of storage carbohydrates in its pads (cladodes) and fruit (tuna), and 2) characterize the epicuticular waxes of Opuntia cladodes and identify coupling of developmental changes in wax composition to an epidermal transcriptome. The major tasks of this project include 1) using next-generation and single-molecule sequencing to sequence the O. ficusindica genome and transcriptome, 2) developing innovative strategies to increase lipid production and storage in Opuntia cladodes via candidate gene modifications, and 3) identifying key compounds in the epicuticular wax of Opuntia under developmental and waterdeficit stress conditions using GC-MS. Sequencing has been completed for Illumina-based RNAseq analysis of plants grown under well-watered and water-deficit stressed conditions and initial results of this analysis will be presented. The genome of diploid O. cochenillifera is also being sequenced by PacBio to generate a scaffold for the octoploid O. ficus-indica genome. Multiple candidates modeled after Arabidopsis genes have been chosen for

oleogenic reprogramming of Opuntia and combinatorial strategies for their use will be outlined. Gene constructs have been designed with constitutive, mesophyll-specific promoters to target lipid production to vegetative tissues. Initial wax data from mature cladodes indicate novel longchain aldehydes that have not been described in other plant species. Preliminary data from cutin analyses will also be presented.

P8 *An assessment of the floristic diversity, life-forms and biological spectrum of Swat Ranizai, District Malakand*
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An inventory of the plant species in Swat Ranizai of District Malakand, Khyber Pakhtunkhwa, Pakistan was made during March 2013 to October 2015. A total of 240 plant species belonging to 85 families and 188 genera were recorded, including five ferns, one gymnosperm, 38 monocots and 196 dicots. Poaceae was found to be the most dominant family in the area that contributed 20 species to the overall floristic composition of the area followed by Asteraceae (14 spp.), Lamiaceae (13 spp.), Papilionaceae (10 spp.) and Solanaceae (10 spp.) respectively. The remaining families shared less than 10 species to the overall floristic composition. It was observed that most of the taxa were perennials (161 spp.) and annuals (73 spp.) whereas biennial were merely 06 species of the total. Based on habit, herbs were most frequent (108 spp.) whereas the representation of grasses and climbers were comparatively high (18 spp.) then sedges (03 spp). The results show that Phanerophytes were the most abundant lifeform (92 spp.) and Microphyll had the most dominant leaf size spectrum in the total floristics. The χ^2 test demonstra significant differences ($\chi^2 = 46.19, p < 0.001$), from normal Raunkiaer's spectrum. It was concluded that the dominance of Phanerophytes and Microphyll leaf size is due to the influence of typical climate of subtropical regions, though receive a significant amount of precipitation and the area is under heavy biotic pressure. The present study will be helpful in the restoration and conservation plans of the ecologically and medicinally important plants in Malakand Division

P9 *Seed yield improvement of the biofuel plant Jatropha curcas through genetic engineering of cytokinin metabolism*
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Jatropha curcas (Jatropha), whose seed content is approximately 30–40% oil, is considered a potential biofuel plant because the composition of its seed oil is suitable for biodiesel and bio-jet fuel production. As a male-biased monoecious perennial species, Jatropha has a low female-to-male flower ratio, which is one of the most important reasons for its poor seed yield. We found that applying 6-benzyladenine (BA), a synthetic compound with cytokinin (CK) activity, to the inflorescence meristems of Jatropha significantly increased the flower number and the female-to-male flower ratio. Furthermore, BA treatments induced bisexual flowers, which were not found in control inflorescence. Consequently, a 3.5-fold increase in fruit number and a 2.3-fold increase in final seed yield were observed in inflorescences treated with 160 mg/L of BA. In addition, treatment of Jatropha with thidiazuron (TDZ), another synthetic CK, also increased the number of female flowers by promoting pistil development, and induced bisexual flowers by reversing stamen abortion during Jatropha flower development, which indicates that the development of both pistils and stamens in Jatropha requires CK. As both female and bisexual flowers influence seed yield, TDZ treatment can significantly increase the number of fruits, and

thus final seed yield. To investigate which genes and signal pathways are involved in the response to cytokinin in Jatropha inflorescence meristems, we further examined the transcriptional levels of genes in Jatropha inflorescence meristems at different time points after cytokinin treatment using the next-generation sequencing technology (Roche 454 sequencing) and a microarray analysis. Differentially expressed genes involved in the metabolism and signaling of cytokinin and other phytohormones, flowering and floral organ development, and cell division were identified. Transgenic approaches to engineer the metabolic pathways of cytokinins in Jatropha inflorescence meristems will be discussed.

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P10 *Lignin Degradation During Rice Lateral Root Emergence*
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Lateral roots expand total root system surface area to substantially increase nutrient and moisture uptake. Cells overlying lateral root primordia (LRP) in Oryza sativa (Os), deform and appear to disassmble during LRP emergence, including the highly autofluorescent sclerenchyma layer, which lies between the cortex and the exodermis. Little is known about the cell wall changes that permit this dramatic restructuring. We report a selective reduction in autofluorescence of sclerenchyma cells that overly the LRP, relative to distal sclerenchyma. The signal reduction occurs when the LRP have several (3-5) cell layers to pass before reaching the sclerenchyma, consistent with extensive cell-to-cell communication. Removal of lignin from root cross sections with 0.1 M HCl and 10% $NaClO_2$ greatly reduces autofluorescence in all sclerenchyma cells. In contrast, immunofluorescence of feruloylated arabinoxylan does not show a signal reduction, and visualization of FA fluorescence at high pH (10.3) is similarly unaltered. These results suggest that lignin is the target of a degradation process that accompanies LRP emergence in rice. Future work aims to identify the enzymes responsible for this process with the long-term goal of creating self-degrading plants for improved biomass-processing efficiency in lignocellulosic biofuel production. Additionally, further understanding of LRP emergence may lead to optimization of lateral root density for improving water and nutrient access by crops.

P11 *Metabolic engineering of p-coumaraldehyde-derived lignin in Arabidopsis thaliana*
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Lignin contributes substantially to the recalcitrance of biomass towards saccharification. In order to circumvent this problem, researchers have focused on altering the lignin polymer through genetic manipulations, although, in a number of cases these resulted in an undesired yield penalty. However, recent findings have shown that by knocking out two subunits (MED5A and MED5B) of the transcriptional regulatory complex Mediator the stunted growth phenotype of plants homozygous for a mutant allele of p-coumaroyl shikimate 3´-hydroxylase (C3´H) can be alleviated. Furthermore, these plants synthesize a lignin polymer almost entirely derived from p-coumaryl alcohol. Plants deficient in cinnamyl alcohol dehydrogenase (CAD) are notable in that they incorporate substantial levels of coniferaldehyde and sinapaldehyde into their lignin. Aldehyde-enriched lignin is a desirable trait, since it leads to greater cell wall digestibility. We tested the hypothesis that by stacking mutations in CADC and

CADD on a med5a/5b c3'h genetic background we would block the biosynthesis of p-coumaryl alcohol, making p-coumaraldehyde available for polymerization into a novel kind of lignin. We have found that these plants (med5a/5b c3'h cadc cadd) are viable and histochemical staining of stems as well as derivatization followed by reductive cleavage (DFRC) suggest that these plants continue to synthesize pcoumaryl alcohol despite being mutated for the CADs typically considered to be required for monolignol biosynthesis. The genome of A. thaliana encodes for 9 CAD isoforms making it possible that another CAD is catalyzing the reduction of p-coumaraldehyde to p-coumaryl alcohol. Based on the data obtained from two independent gene expression experiments we identified two candidates that could be responsible for this catalysis. Therefore, we are using the CRISPR/Cas9 system to knockout these genes in med5a/5b c3'h cadc cadd plants with the expectation that this will be sufficient to block p-coumaryl alcohol synthesis.

P12 *Overexpression of MYB transcription factors increase glucose release by enzymatic hydrolysis in sugarcane.*

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Lignocellulosic ethanol, produced by the fermentation of sugars from the plant cell wall, is a promising alternative to fossil fuels. Reduction of lignocellulose recalcitrance to biological conversion would have a significant effect on ethanol production by reducing the cost of biomass pretreatment. Sugarcane bagasse is an abundant natural lignocellulosic residue that remains after extraction of juice from the sugarcane stalk. A large amount of bagasse is currently burnt as a low-grade fuel for energy recovery. Production of biofuels from this abundant lignocellulosic bagasse would boost the potential ethanol output from sugarcane per land area. Towards this goal, we overexpressed two maize transcription factors – ZmMYB31 and ZmMYB42 with and without UTR sequences in sugarcane. Plant height and number of internodes were not significantly influenced by overexpression of either of the transcription factors. Genes in the lignin pathway were significantly downregulated by both transcription factors, with MYB31 downregulating more lignin genes than MYB42. Of the MYB31 expressing sugarcane only three lines showed a significant decrease in total lignin content, whereas in MYB42 expressing plants six lines showed a significant decrease in lignin content. There were no significant changes in structural carbohydrate contents in the majority of MYB42 plants. All MYB42 plants further analyzed showed significant increases in glucose release by enzymatic hydrolysis while only two MYB31 plants released more glucose than control plants. This correlated directly with a significant decrease in acid insoluble lignin. Soluble sucrose content of the MYB42 transgenic plants did not vary compared to control plants, while it decreased in two lines of MYB31 transgenic plants. Our results indicate that MYB42 can be successfully used to reduce lignin content without affecting sucrose and biomass content.

P13 *VIGS-Mediated screening of secondary cell wall genes for identifying targets for genetic improvement of saccharification efficiency in bioenergy plants*

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Lignocellulosic secondary cell walls (SCW) provide essential plant materials for the production of second generation bioethanol. Therefore, thorough understanding of the process of SCW formation in plants is beneficial for efficient bioethanol production. Recently, we provided the first proof-of-concept for using Virus Induced Gene Silencing (VIGS) approach for rapid functional characterization of nine genes involved in cellulose, hemicellulose and lignin synthesis during SCW formation. Here we report VIGS

mediated screening of about 38 genes of unknown function that was followed by the functional characterization of two genes involved in SCW formation. Stems of VIGS plants silenced for genes #29 (DUF579) and #35 (KNAT7) showed increased amount of xylem formation but thinner cell walls than controls. These results were further confirmed by production of stable transgenic tobacco plants manipulated in expression of these genes. Stems of stable transgenic tobacco plants silenced for these two genes showed increased xylem proliferation with thinner walls whereas transgenic tobacco plants overexpressing these two genes showed increased fiber cell wall thickness. The two genes, NbDUF579 gene is involved in xylan synthesis and NbKNAT7 transcription factor (TF) family gene is involved in positive regulation of SCW formation, respectively. Glycome analyses of cell walls showed increased polysaccharide extractability in 1M KOH extracts of both VIGS- NbDUF579 and NbKNAT7 lines suggestive of cell wall loosening. In addition, VIGSNbDUF579 and VIGS-NbKNAT7 lines showed increased saccharification rates (74.5% and 40% higher than controls, respectively). A meta-analysis of currently available data on genetic engineering of plants for improved saccharification efficiency (SE) will also be presented. SE is one of the highly desirable traits for producing higher quantities of bioethanol from lignocellulosic materials of bioenergy plants.

P14 *Integrating energy, food, and waste management systems for environmentally sustainable bioenergy production*
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Methane emissions are a growing concern for greenhouse gas (GHG) management. Anthropogenic methane emissions are primarily from energy, agriculture, and waste management. In the United States 30-32% of anthropogenic methane comes from oil and gasoline use and 21-22% of emissions come from landfills. Yet, there are opportunities for converting landfill methane emissions into fuel (that could displace oil and gasoline use) by integrating waste management with bioenergy production systems. Anaerobic digestion is a bioenergy production pathway that converts wastes to methane fuel (biogas). In some cases, a by-product of this system is organic fertilizer that can support both food and bioenergy agriculture. Advanced bioenergy systems at the commercial scale are few in the United States, and are mostly reliant on uniform format feedstocks. Yet, non-uniform format wastes represent a tremendous opportunity as bioenergy feedstocks. Anaerobic digestion for biogas is a recentlyadopted technology in the U.S., and life-cycle assessments (LCAs) of biogas production have been parameterized comparably to other bioenergy pathways, with broader system boundaries included in the assessments than are typical in other energy production systems. One interesting difference in biogas LCAs that distinguish them from LCAs of conventional agriculture and other bioenergy systems is that waste streams are consistently internalized and the system boundary for biogas production is often inclusive of another agricultural production system or municipal waste treatment system. In other words, anaerobic digestion can be integrated with other bioenergy production systems and has the potential to promote efficient agricultural management. Improved waste and fertilizer management will in turn decrease GHG emissions and spare land for other uses. As such, anaerobic digestion systems can address multiple environmental and economic challenges related to energy, agriculture, and fertilizer management.

P15 *Modifying lignin content and composition to improve sorghum for bioenergy*
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Modifying lignin content and composition are major targets for bioenergy feedstock improvement for both cellulosic and thermal bioenergy conversion. Sorghum (Sorghum bicolor) is currently being developed as a dedicated bioenergy feedstock. To increase the energy content of sorghum biomass for thermal bioenergy conversion processes, including pyrolysis and direct combustion, a series of transgenic events ectopically expressing ten monolignol biosynthetic genes and a Myb transcription factor, SbMyb60, were developed. Higher lignin content is desirable, because lignin has a greater energy content than cellulose or hemicellulose cell wall components. The events overexpressing SbMyb60 have elevated levels of proteins from the lignin biosynthetic pathway, increased levels of phenolic compounds and increased energy content. This result indicated that overexpression of this transcription factor is sufficient to induce phenylpropanoid synthesis in sorghum. Likewise, overexpression of caffeoyl CoA O-methyltransferase (SbCCoAOMT) resulted in increased energy content, but without increasing lignin concentration. In contrast, reducing lignin content is desirable for improving cellulosic bioenergy conversion. To reduce lignin content and alter lignin composition, brown midrib (bmr) mutants are being utilized. bmr6 and 12 sorghum lines have been previously shown to significantly increase ethanol conversion through saccharification and fermentation. Currently, five other bmr loci are being evaluated for the potential to improve biomass conversion. In addition, both the bmr and the transgenic strategies are being combined together with the goal of tailoring lignin content and composition beyond what has been previously observed in sorghum. The development of these experimental lines with altered phenylpropanoid metabolism will lead to a greater understanding of how these modifications impact plant fitness and affect bioenergy conversion technologies in sorghum and other bioenergy grasses.

P16 *New Strategies for Improving the Water-use Efficiency of Bioenergy Feedstock Production*
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More than 40% of the world's land area is considered arid, semi-arid, or dry sub-humid, with precipitation rates inadequate to support conventional bioenergy crops. The rapid adoption of crassulacean acid metabolism (CAM)-performing biofuel feedstocks (e.g., Agave, Opuntia spp.) with greater drought durability and water-use efficiency (WUE) could permit sustainable biomass production using only 20% of the water needed to produce traditional bioenergy crops. Alternatively, the introduction of CAM machinery into C3 plants might also confer improved WUE. Tissue succulence allows plants to avoid drought under water-limiting conditions and is often correlated with the optimal performance of CAM. Thus, tissue succulence might be a key anatomical trait to engineer for enhancing the efficiency of engineered CAM by increasing malate storage capacity, reducing intercellular air space (IAS) to limit the diffusion of CO2 out of the leaf during the day, and thereby enhancing the potential for refixation of CO2 by RUBISCO. Engineered tissue succulence in Arabidopsis was achieved by overexpressing a modified basic helix-loop-helix (bHLH) transcription factor from Vitis vinifera. Arabidopsis plants engineered for enhanced tissue succulence showed up to a 2.2-fold increase plant leaf fresh weight and up to a 2.4-fold increase in leaf and root dry weight, and seed production relative to controls. The increased size of all organs also resulted in up to a 1.6-fold increase in leaf thickness, a 1.8-fold increase in leaf succulence, a 2.9-fold increase in leaf water content, and a 37% reduction in intracellular air space. Importantly, plants with engineered succulence exhibited significantly increased seed number per silique, seed area, seed weight, and overall seed yield. Lastly, the engineered succulence plants displayed greater water-use efficiency, and salinity and water-deficit stress tolerance, likely due to their ability to retain and store water and solutes within their tissues.

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P17 *Mechanistic studies of glycoside hydrolases in reducing cell wall recalcitrance*
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The overall recalcitrance of lignocellulosic biomass to hydrothermal pretreatment and subsequent enzymatic deconstruction represents a significant cost barrier to the widespread development of biofuels technologies. To explore the possibility of reducing the recalcitrance of lignocellulosic biomass, we have generated a library of glycoside hydrolases across several families from a variety of sources, which we transformed into Arabidopsis thaliana with expression targeted to the cell wall under a constitutive promoter. Here we explore the possibility that in planta expression of specific glycoside hydrolase families will allow these enzymes to access their substrates during cell wall construction, rendering cellulose more amenable to pretreatment and enzyme digestion. The transgenic A. thaliana plants were healthy and developed normally compared with the wild type. After hydrothermal pretreatment and enzyme digestion, certain transformed plants were 10-15% more digestible than the wild type plants, suggesting that the expression of specific GH's during cell wall construction altered the inherent recalcitrance of the cell wall. We have also elucidated some of the mechanisms of GH5 mediated plant cell wall recalcitrance reduction.

P18 *Towards identifying the bottlenecks of oil synthesis in pennycress (Thlaspi arvense L.) embryos, a source of renewable fuel*
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Pennycress (Thlaspi arvense L.), is a promising source of biodiesel and aviation fuel due to the oil composition in its seeds. However, for this plant to become an economically viable bioenergy crop, its oil production needs to be improved. In pennycress embryos, fatty acid synthesis (FAS) requires carbon skeletons, energy, and reducing power; all of these are provided by central metabolism. We hypothesize that one or more steps is/are limiting FAS. To test this hypothesis, we have previously conducted a metabolomics study that identified the active biochemical pathways during FAS in pennycress embryos. We are now performing 13C-Metabolic Flux Analysis to quantify in vivo carbon fluxes through each metabolic pathway, which will pinpoint potential bottleneck(s). First, this approach requires establishing in vivo culture conditions that mimic the development of pennycress embryos in planta. Given that substrates furnished by the plant are unloaded in the endosperm liquid and taken up by the embryo, the endosperm composition was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to design the culture growth medium. Second, the efficiency with which pennycress embryos convert carbon into biomass was measured to be 60%, which is lower than other Brassicaceae, and reinforces our hypothesis that there are bottlenecks in FAS. Third, pennycress embryos were incubated with 13C-labeled substrates until metabolic and isotopic steady state. The resultant labeling in intracellular metabolites was quantified by LC-MS/MS. The main findings from the 13C-glutamine labeling experiment showing no occurrence of gluconeogenesis and reversibility of Krebs cycle will be presented and discussed. Future plans include the incorporation of all the labeling information into a mathematical model to generate a flux map, which will identify the bottleneck(s) in FAS. Understanding

the biochemical basis of oil synthesis in pennycress embryos is fundamental to advance future breeding and/or metabolic engineering efforts aiming at increasing FAS.

P19 *The environmental productivity and photosynthetic light response of the CAM plant Agave americana (L.): a potential semi-arid biofuel feedstock*
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The potential for the desert succulent species Agave americana (L.) as an advanced biofuel crop in water limited regions has recently been recognized. However, the potential productivity of A. americana in the United States is not yet fully understood. This study developed an environmental productivity index (EPI) model that can be used to estimate the actual growth of A. americana based on the seasonal patterns of water, temperature, and photosynthetically active radiation (PAR) on a monthly time scale for any given region. Previously published research was used to construct indices that predict growth responses of A. americana to water and temperature. Light responses, however, have not previously been determined for this species, and this study is the first to experimentally resolve the physiological response of A. americana to varying intensities of PAR. The photosynthetic response to light was determined by measuring gas exchange over 24 hours in plants that were acclimated to varied light levels over 10 days. Results were used to derive a predictive index of the growth response to light. Maximum CO2 fixation rates were observed at a light intensity of 1250 µmol photons m-2s-1. A monthly EPI was calculated as the product of the water, temperature, and light indices appropriate for the monthly environmental conditions in Maricopa, AZ, where the first trial of A. americana was recently completed. Growth predicted using the EPI was compared to actual production. The summed EPI values were highly correlated (R2 = 0.99) with the average total biomass of healthy 2 and 3 year old plants. Quantitative relationships derived here between environmental conditions and production of A. americana provide a simple tool to estimate and compare potential productivity across regions where this species has not yet been grown, and to determine potential geographic ranges in the future as climate changes.

P20 *Quantitative Plant Science Initiative (QSPI): A team based BNL capability to accelerate fundamental plant science towards predictive design of bioenergy crops*
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The Quantitative Plant Science Initiative (QPSI) is a new BER-aligned capability based at Brookhaven National Laboratory. The capability combines crossdisciplinary expertise with state of the art technologies to accelerate foundational understanding of plant biology. The availability of whole-genome sequences has ushered in a new era of biological research. With these invaluable resources in hand we can appreciate the extent of biological complexity and use genome sequences to assess our progress and lack of knowledge. For instance, even in very well studied model organisms, over 40% of genes are of unknown function. In less characterized complex organisms, such as bioenergy crops, up to 80% of all genes in a given genome are of unknown or very limited function. Indeed, a complete functional understanding (i.e. combined knowledge of biochemical activity, biological role and compartmentalization) is missing for ~95% of plant genes. This fundamental knowledge gap undermines the ability of systems scientists to realize the potential of genomic science and impedes our ability to leverage photosynthetic organisms to meet national energy needs. QPSI is addressing these challenges

by reducing gene function uncertainty to provide fundamental knowledge for plant bioenergy production. Accelerated and highly scalable analyses towards this aim are made possible by combining experimental miniaturization with high-throughput (HTP) laboratory automation approaches for phenomics, analytical chemistry, imaging, and structural determination with targeted HTP validation. A core tenet of our approach is to employ precisely defined standardized conditions enabling direct comparison and interpretation of datasets to strengthen their value in modeling biological systems. To accomplish these goals we are initially employing unicellular photosynthetic organisms such as the flagship Chlamydomonas reinhardtii, and utilizing computationally derived orthology relations for the transfer of knowledge to other flagships and bioenergy crops via established links with PlantSEED and KBase for subsequent in situ application.

P21 *Effective Copper-Catalyzed Alkaline-Oxidative Pretreatment of Woody Biomass*
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Strategies to improve copper-catalyzed alkaline hydrogen peroxide (Cu-AHP) pretreatment of hybrid poplar were investigated that both increased hydrolysis yields while simultaneously decreased process inputs through (i) more efficient utilization of H2O2 and (ii) the addition of an alkaline pre-extraction step prior to the metal catalyzed AHP pretreatment. Our results revealed that the alkaline pre-extraction step improved both lignin and xylan solubilization, which ultimately led to improved glucose (86%) and xylose (95%) yields following enzymatic hydrolysis. An increase in the lignin solubilization was also observed with fed-batch H2O2 addition relative to batch-only addition, which again resulted in increased glucose and xylose yields (77% and 93% versus 63% and 74%, respectively). Importantly, combining these strategies led to significantly improved sugar yields (96% glucose and 94% xylose) following enzymatic hydrolysis. In addition, we found that we could substantially lower the chemical inputs (enzyme, H2O2, and catalyst) while still maintaining high product yields utilizing the improved Cu-AHP process. This pretreatment also provided a relatively pure lignin stream consisting of ≥90% Klason lignin and only 3% xylan and 2% ash following precipitation. Two dimensional heteronuclear single-quantum coherence (2D HSQC) NMR and size exclusion chromatography demonstrated that the solubilized lignin was high molecular weight (Mw ≈22,000 Da) and only slightly oxidized relative to lignin from untreated poplar. This study demonstrated that the fed-batch, two-stage Cu-AHP pretreatment process was effective in pretreating hybrid poplar for its conversion into fermentable sugars. Results showed sugar yields near the theoretical maximum were achieved from enzymatically hydrolyzed hybrid poplar by incorporating an alkaline pre-extraction step prior to pretreatment and by efficiently utilizing H2O2 during the Cu-AHP process. Significantly, this study reports high sugar yields from woody biomass treated with an AHP pretreatment under mild reaction conditions.

P22 *Generation of Neo Octaploid Switchgrass*
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Switchgrass (Panicum virgatum L.) exists as multiple cytotypes with octaploid and tetraploid populations occupying distinct, overlapping ranges. These cytotypes tend to show differences in adaptation, yield

potential, and other characters, but the specific result of whole genome duplication is not clear and 8x and 4x switchgrass populations are reproductively isolated with limited genetic exchange. To create new opportunities for population improvement and to study the effects of whole genome duplication on switchgrass, seedling treatment of the tetraploid cultivar 'Liberty' with microtubule inhibitors was used to generate an octaploid population. Resulting octaploids, tetraploids, and cytochimeras were resolved by intercrossing octaploid sectors to produce a population of 19 octaploid families. Fertility of octaploid sectors was significantly reduced relative to tetraploid sectors and caryopsis size significantly increased. Cell size was significantly increased which resulted in quantitative changes to leaf anatomy. During seedling and early vegetative growth stages, no differences in vigor or tillering ability were seen. This technique resulted in efficient genome doubling and was simple to perform. However, aneuploids were also identified with both larger and smaller than expected genome sizes.

P23 *A Multilevel Approach Applied to Sugarcane Root Aerenchyma Development*
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Systems can be defined as structures that are formed by elements that interact giving rise to a meaning of function. In biology, understanding how systems function requires knowing what elements form systems, how these elements interact with each other in order to give rise to the emerging biological function of the system. Biological systems perform their functions by integrating actions at different scales that lead to the emerging overall function of the system. It is now clear that every biological process will be funded on mechanisms related to the scales of genome, transcriptome, proteome, metabolome and phenome. In the literature, these scales are usually studied separately, i.e. the biological function is usually explained by the individual mechanisms associated to one or two of the scales . The present work tries to evaluate all the scales mentioned above in an integrated form so that a much wider systems approach can be visualized. Here we used as a model a process of modification of cell walls within the roots of sugarcane. During development of the roots, the tip of the root contains cells that are dividing and producing cell differentiation through a complex network of signals that lead to cell expansion and elongation with the concomitant formation of the vascular system. At the same time, the cortex undertakes changes in cell walls that resemble degradation. In both cases, development is associated with programmed cell death and cell wall modifications that lead to the emerging functions of nutrient and water transportation and oxygen access to the living cells through a structure named aerenchyma that forms in the cortex. We have concurrently analyzed transcriptomics, quantitative proteomics, metabolomics and phenomics of the developmental process of sugarcane roots. Integration of the data afforded the discovery of a collection of key genes related to the mechanisms associated to the systems mentioned above. The integrated view of events occurring at the same time was collapsed to single map that describes wall-related events during aerenchyma formation system at different scales at the same time. After having all these information analyzed, we are now putting together a computational tool that can help visualization of the whole system. We expect that with this new tool, it would be possible to design modulations of the whole system in much more precise and reliable ways so that plant engineering for cell wall hydrolysis can help development of technologies for bioenergy production from grasses.

P24 *Hepatoprotective and antioxidant properties of Picrorhiza kurroa hairy roots extracts on CCl4 induced liver toxicity in hepatic cell line.*

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Transgenic hairy root clone of Picrorhiza kurroa (Scrophulariaceae) with high biomass and iridoid glycoside content was cultured in liquid MS medium. It offers good prospects for the development of an effective alternate production source of the raw materials for the commercially important root drug "Picroliv". The aqueous hairy root extract (picroliv) of Picrorhiza kurroa was investigated for its hepatoprotective and antioxidant effects in cell line. Biochemical marker enzymes such as Aspartate aminotransaminase (AST), Alkaline aminotransaminase (ALT), Alkaline phosphatase (ALP) and bilirubin were measured. Further, the antioxidant defense enzymes like Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione-S-transferase (GST) were also estimated. The rhizome extract showed significant ($P<0.05$) hepatoprotective effect by decreased the biochemical marker enzymes level in serum and the antioxidant enzymes were significantly ($P<0.05$) increased when compared to the CCl_4 induced control groups. In this study, we have evaluated the protective effect of picroliv (a purified iridoid glycoside fraction from roots of Picrorhiza kurrooa with hepatoprotective, anti-inflammatory and antioxidant properties) against hypoxic injury by examining lactate dehydrogenase (LDH) release in Hep 3B and Glioma cells. These findings suggest that in vitro cultured hairy root derived picroliv may act as a protective agent against hypoxia/reoxygenation induced injuries, and the underlying mechanism may involve a novel signal transduction pathway.

P25 *The DOE Systems Biology Knowledgebase: A cyberinfrastructure for sharing and integrating data and analytical tools to accelerate bioenergy feedstock research*
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The U.S. Department of Energy Systems Biology Knowledgebase (KBase, http://kbase.us) aims to provide a large-scale computational environment to meet the key challenges of systems biology: predicting and ultimately designing biological function. KBase supports the sharing and integration of reference and experimental data with analysis tools that enable researchers to design computational experiments, test hypotheses, and share findings that can be easily reproduced and extended by other researchers.

Capabilities in KBase that are useful for plant research include gene expression profiling and metabolic network modeling. For example, users can interactively run an RNA-seq pipeline and downstream analysis tools to quantify expression from the RNA-seq reads and thereby identify differential expression between tissues, developmental stages, environmental conditions and genetic backgrounds. KBase also has tools for metabolic model reconstruction and flux balance analysis simulation. These can be used to provide insight into potential metabolic pathways and interactions between plants and microbes, such as the identification of biochemical reactions active in biomass production.

KBase enables users to upload their own data and access public data in KBase to be used in customized,

sequential analyses that target their specific systems biology hypotheses. These computational experiments or analyses are captured in dynamic, interactive documents called Narratives and promote collaboration and reproducibility of scientific results. In addition to data and analysis steps, the Narratives can include images, notes, and links. They can be kept private, shared with colleagues and collaborators, or made public for the benefit of the wider research community.

Visit http://kbase.us/tutorials/ to explore various tutorials related to Expression Profiling and Metabolic Modeling, and to learn how KBase apps might be useful in your research.