



**China-US Workshop on Biotechnology of Bioenergy Plants
November 16-17, 2009
Knoxville, Tennessee USA**

**BIOS AND ABSTRACTS FOR SPEAKERS
POSTER ABSTRACTS**

(in alphabetical order by speaker – poster abstracts follow)

BROWN, WILLIAM F. (BILL)

Bio: Dr. Bill Brown is Dean and Director of UT AgResearch. He received a BS degree from University of Florida, a MS degree from University of Tennessee in Animal Science, and a PhD from the University of Nebraska in Animal Science, Ruminant Nutrition. He spent 26 years at the University of Florida as a faculty member at both campus and Research Center Locations. His research focus is in the area of forage evaluation, protein and energy supplementation programs for beef and dairy cattle; and heifer development programs.

Dr. Brown spent nine years as Assistant Dean and Assistant Director of the Florida Agricultural Experiment Station; also served as Director of Sponsored Programs

BRUCE, BARRY D.

Abstract: *Bioengineering of Photosynthesis: Putting PSI to Work*

Nature has developed remarkable means for harvesting solar energy to drive the process of photosynthesis. In part the remarkably high quantum efficiency associated with photosynthesis, has been enabled by the successful “division of labor” associated with this process. Specifically, in nature, organisms have evolved largely separate biomolecular structures that have become specialized to either **1)** capture photons and facilitate energy transfer via high-efficiency exciton coupling of pigments and **2)** a separate structure, known as a reaction center, to convert this exciton into a charge separation that has a quantum yield approaching unity. Interestingly, the light harvesting process has been very adaptive to capture a wide range of solar energy (370 ⇒ 700 nm Although both the pigments and organization of this light-harvesting complexes demonstrate tremendous diversity, the charge separation process is restricted to only two different complexes know as Photosystem II & I. These photosystems contain a core of only 6 pigments that functions as a reaction center. Drawing on the remarkable efficiency, stability, and renewability of these biological complexes, we have begun to investigate their ability to function as both solar luminescent concentrators, as well as photovoltaic devices that can launch electrons into either organic semiconductors such as C60 or Alq3, or into inorganic semiconductors such as TiO₂ and ZnO nanotubes. I will report on the design and fabrication of these biohybrid PV devices and solar concentrators. In addition, I will discuss future designs to further enhance their EQE towards the goal of a truly sustainable and environmentally benign new design of photovoltaic materials. This work has been supported by grants from Intel, the NSF NIRT program and UTK SEERC.

Bio: Dr. Barry D. Bruce is Professor in the Department of Biochemistry, Cellular and Molecular Biology. He is also an Adjunct Professor of Microbiology and Chemical and Biomolecular Engineering. He is a Founding Member and the Associate Director of the Sustainable Energy and Education Research Center (SEERC). Dr. Bruce has B.A.s in both chemistry and biology from the University of California at Santa Cruz and holds a MS in Biochemistry/Biophysics from the University of Massachusetts at Amherst. In 1990, he received his Ph.D. in Molecular Plant Biology from the University of California at Berkeley and joined the UT Knoxville faculty in 1994 after completing a National Science Foundation post-doctoral Fellowship in Plant Biology at the University of Wisconsin at Madison.

Professor Bruce's laboratory works on two aspects related to photosynthesis: 1) one group investigates how proteins are targeted and transported to chloroplasts in plant cells. This work is not only fundamental to how plants grow and perform essential processes such as photosynthesis, but has also led to discoveries that enable scientists to engineer plants with higher nutritional content and to grow edible vaccines to help fight the spread of disease. A second group in Dr. Bruce's lab is developing new methods to apply the process of photosynthesis towards new energy sources such as hydrogen and electricity. This work attracted the attention of Forbes Magazine which recently recognized Dr. Bruce's as one of the "Ten Revolutionaries that May Change the World." A prolific researcher, Dr. Bruce has produced a large and important body of work contributing to both of these research fields. His work has been reported in *The Plant Cell*, *Trends in Cell Biology*, *Plant Physiology*, *EMBO Reports*, *Molecular Biology of the Cell*, *J. Biological Chemistry*, *Nano Letters* and *Biochemistry*.

Dr. Bruce teaches undergraduate and graduate courses in topics including Plant Physiology, Advanced Cell Biology, and Advanced Protein Chemistry and has been recognized several times for educational mentoring and outreach. Bruce's research is supported by grants from the National Science Foundation, USDA and DOE. He is currently the PI of a prestigious \$1.7 million NIRT (Nanoscience Interdisciplinary Research Team) award from NSF to lead a team of researchers who are trying to harness the power of photosynthesis to generate electricity from solid-state solar panels. In 2008, he was a co-PI of a \$3.1 million IGERT Award from NSF develop a graduate program in Sustainable Technology through Advanced Interdisciplinary Research (STAIR).

Dr. Bruce's work has been recognized on campus with awards and honors, as well as at the national and international levels. The Faculty Senate Research Council & Office of Research has twice awarded him the Scholarly Activity and Research Incentive Funds Award, and he won the Science Alliance Research Excellence Award three times. Bruce has been invited to give many national and international presentations. In 2007 he was invited to be one of the EMBO/FEBS Lecturers on The Molecular and Cellular Biology of Membranes held in Corsica, France.

CHEN, FENG

Abstract: *Uncovering the Terpenoid Machinery for Biofuel Production—*

Terpenoids constitute a very diverse class of secondary metabolites. Many organisms, especially plants, make terpenoids in large quantities. A fraction of fossil fuel was derived from terpenoids. Because of their hydrocarbon nature, terpenoids are an attractive source of biofuel, especially biodiesel. In this talk, the chemical properties of terpenoids and the potential of using terpenoids as a form of renewable biodiesel will be introduced. Next, our current understanding of terpenoid biosynthesis and genomic approaches that can be used for elucidating the molecular machinery of terpenoid biosynthesis will be presented. At last, novel strategies of using metabolic engineering to create novel bioenergy crops based on the terpenoid pathway will be discussed.

Bio: Dr. Feng Chen received his Ph.D in Plant Biology from the University of California, Davis and did postdoc training at the University of Michigan. Dr. Chen joined the Plant Sciences faculty at the University of Tennessee since September 2004. Research in the Chen group is directed towards understanding plant secondary metabolism. Despite the importance of secondary metabolites for

plants and humans, plant secondary metabolism in general remains poorly characterized. The research program in the Chen group employs an integrated functional genomic approach, which combines bioinformatic analysis, metabolic profiling, expression profiling, and large-scale in vitro biochemical assays, to systemically discover novel genes in plant secondary metabolism in various plant species. This study is expected to yield important results that will open new avenues to understanding the metabolism, physiology and evolution of plants, and provide knowledge and tools for crop improvement, including bioenergy crops, using metabolic engineering.

ENGLISH, BURTON

Abstract:

Bio: Dr. Burton C. English, an Agricultural Economics professor at The University of Tennessee, develops and uses simulation and programming models to evaluate production and environmental issues associated with agricultural production. Involved in examining the economics of biomass to energy conversion for nearly 30 years, he has analyzed alternative environmental policies and impacts on production agriculture, examined alternative land use changes on net farm income, estimated the impacts of environmental policies on small and medium-sized agricultural producers, and projected the impacts of changes in the agricultural industrial complex and the corresponding changes to occur in the region/nation's economy. He has, with his colleagues, conducted recent national analyses for 25 X '25, the Governors Ethanol Coalition, Department of Energy, and USDA. These analyses are long term in nature evaluating impacts 15 to 25 years into the future.

In addition, Dr English co-led a project that established 124 acres of switchgrass on both farmers and Experiment Station lands. On these acres, variety trials, along with fertilization and seeding rate experiments are being conducted on a variety of landscapes. Once the switchgrass is harvested, two conversion technologies are currently underway. Bio-oil conversion of switchgrass is underway to evaluate the impact moisture, particle size, and conversion temperature has on the quality of bio-oil. Combustion of switchgrass is also taking place at the Gadsden, Alabama power plant with measurements on the change in emissions that occur as a result of co-firing the switchgrass with coal. Currently, Dr. English is evaluating the impacts of switchgrass establishment as a dedicated energy crop on carbon storage.

RANDALL W. GENTRY

Bio: Dr. Randy Gentry is the Director of the Institute for a Secure and Sustainable Environment at the University of Tennessee, President of the University of Tennessee Research Foundation, and an Associate Professor in UT's Department of Civil and Environmental Engineering. Gentry, who earned his PhD in civil engineering from the University of Memphis, also directs the Southeastern Water Resources Institute, a multidisciplinary, multi-institutional research entity devoted to the study of science, technology, and public policy issues related to surface and ground water. Gentry's research focuses on the evaluation of groundwater hydrologic interfaces of highly localized systems as well as broad-scale watershed processes. Under Gentry's direction, ISSE's multidisciplinary research team is exploring the convergence of the carbon cycle and carbon sequestration, climate and natural systems response, and renewable bioenergy.

JACKSON, SAMUEL

Abstract: *Purpose Grown Feedstocks: A Focus of the University of Tennessee*

The University of Tennessee has placed considerable emphasis on the development of biomass-based bioenergy, including liquid fuels, bioproducts, and biopower. In recent years, both the Tennessee and United States governments have made an unparalleled commitment through investment to lead the transition to an advanced biofuels economy with a commitment to support the development of dedicated bioenergy crops, the related supply chain and the development of a biomass-based industry.

As we expand our reliance on biomass-based materials, expectations are that agricultural crop residues and forest residues will be the first feedstock sources for the biorefining industry. Energy crops will develop and become integrated into the agricultural market as the biorefining industry matures and creates the demand for those resources. However, the emergence of this new industry hinges on the availability of an affordable, stable, and sustainable supply of lignocellulosic biomass with relatively consistent performance characteristics. This will not happen without a concerted effort to optimize feedstock production systems with improved crops offering higher yields of biomass, and biomass that is more readily converted into liquid fuels, chemicals, and power. The University of Tennessee is working closely with the US Department of Energy, the Sun Grant Initiative, and other private partners to build, optimize and commercialize large-scale biomass production systems. The University and its partners are working in a variety of areas, including feedstock production, transportation and logistics, and feedstock availability assessment and modeling.

The development of the cellulosic biofuels supply chain, from biomass to fuels and products, will have a significant impact on the energy future of the state and nation. These new systems, technologies, and products will provide increased economic opportunities for rural economies, increased energy security for the nation, and improved environmental impacts of energy production/utilization.

Bio: Samuel W. Jackson is a Research Assistant Professor with the University of Tennessee Agricultural Experiment Station, Office of Bioenergy Programs. He also serves as Vice President for Feedstock Operations of Genera Energy LLC, a subsidiary of the UT Research Foundation. Sam received his Bachelor of Science degree in Wildlife and Fisheries Science and his Master of Science in Forestry from the University of Tennessee. He earned a Doctor of Philosophy in Natural Resources in 2008.

Sam works with a variety of programs, including the UT Biofuels Initiative (<http://www.UTbioenergy.org>) and the Southeastern Sun Grant Center (<http://sungrant.tennessee.edu>). The Biofuels Initiative is a project that seeks to promote the development of cellulosic ethanol in Tennessee and the nation. The Southeastern Sun Grant center is a federally funded program designed at facilitating research in biomass and bioenergy at Land-Grant universities around the Southeast. UT coordinates the program for the region. Sam is responsible for a variety of tasks in the Center, including coordination of the competitive grants programs.

With Genera Energy, Sam is responsible for developing all aspects of the feedstock program. Sam works directly with private landowners, UT Extension, and others to create a sustainable feedstock supply chain. Sam also works with developing alternative energy crop markets with the ultimate goal of attracting commercial investment in Tennessee.

Sam is a member of the Society of American Foresters and the Tennessee Forestry Association. Originally from West Virginia, Sam lives in Seymour, TN with his wife Daphne and son Noah.

LI, LAI-GENG

Abstract: *Dissection of Cellulose Synthase Complex in Populus* (with Dongliang Song, Junhui Shen, and Laigeng Li)

It is generally believed that the synthesis of cellulose in higher plants is catalyzed by cellulose synthase complexes (CSCs) localized on the plasma membrane. In our recent study, we isolated and analyzed CSCs from the differentiating xylem tissue of *Populus*. Immunoprecipitation using antibodies specifically recognizing various CESA proteins led to the identification of at least two types of CSCs in the membrane protein fraction of the xylem tissue. Proteomic analysis further confirmed that the isolated CSCs were assembled from different CESA protein constituents. Immunolocalization results confirmed that both types of CSCs are involved in the secondary cell wall formation of the xylem cells. These results suggest that CSCs, deliberately composed of different CesAs, may be involved in regulating cellulose formation in xylem cells. In addition, a number of

non-CesA proteins were also identified to be associated with the isolated CSCs indicating that the CSC machinery may be aided by other proteins in addition to CesAs.

Bio: Professor Lai-Geng Li has been studying cell wall biosynthesis for more than 14 years. His research interest focuses on the molecular biology and genomics of cell wall formation, including lignin, cellulose and hemicellulose biosynthesis. His research has led to elucidation of the monolignol biosynthetic pathways in poplar and provided important insights into understanding of evolution of lignin biosynthesis pathways during the process of plant evolution. Professor Li earned his Ph.D. degree in molecular genetics from Michigan Technological University of USA in 1997. Before he joined the Chinese Academy of Science, he was working at Michigan Technological University as a research assistant professor and at North Carolina State University as an associate professor.

LIU, GONG-SHE—Workshop Co-Chair

Abstract: *Analysis of transcriptome in Leymus chinensis using 454 Life Sciences Technology* (with Gongshe Liu, Xiaofeng Li, Shuangyan Chen, Xingyong Ma, Dongmei Qi, Xianjun Peng, and Xiaoxia Li)

Leymus chinensis (Trin.) Tzvel., a perennial rhizome grass, has a very broad distribution in China. It grows across diverse soil and climate conditions with strong tolerant to diverse environmental stresses such as cold, salt and drought. Therefore, it is very useful to obtain important genes of this species for improving bioenergy plants and enable them grow well in marginal soil and bad environmental conditions. Because of limited genomic and EST information of *L. chinensis*, the transcriptome sequencing was performed using 454 Life Sciences technology. Total 421,180 raw reads were generated with an average length of 256bp. Among these reads, 374,510 (89.1%) reads were assembled into 25,897 contigs with an average length of 455bp and a maximum length of 3,719bp, 45,668 (10.8) reads were singletons. A total of 71,565 unique sequences were obtained and 65,584 putative open reading frames (ORFs) could be predicted. The functional classifications of the ESTs were carried out using the Gene Ontology (GO) classification system. BLASTX analysis at E-value of 1e-5 showed that there were more *L. chinensis* cDNA sequences similar to rice, Wheat, Barley than Arabidopsis. 1,200 (1.7%) ESTs were predicted to 42 transcription factor families. The ESTs resources will provide a basis for gene discovery and the determination of gene function in this species.

Bio: Prof. Gongshe Liu got his Ph.D. degree from Blaise-Pascal University and CNRS of France in 1986. After that, he started his post-doctoral research, and since then has been working at the Institute of Botany, Chinese Academy of Sciences. Now he is Director Assistant of R&D Center for Energy Plants in IB-CAS.

Prof. Liu has been studying *L. chinensis* over fifteen years. His group collected 548 accessions of *L. chinensis* from China, Mongolia and Korea. These materials had a wide range of genetic variation. Seventeen elite germplasm were obtained, among them, 6 populations are going on variety experiment. He found that *L. chinensis* was a self-incompatible species; however, hybrid seed-set rate was high. He revealed the parent source of *L. chinensis* and established an efficient regeneration and genetic transformation system. His current research is focused on comparative genomics and corresponding techniques to select new genes from *L. chinensis* related with high bio-energy and adverse resistance as well as genes regulation, which will provide the fine gene resources for the molecular design breeding of sweet sorghum and other bioenergy plants. He is the correspondence scientist of 973 key project for gene cloning and functional analysis of native grass in China (2007-2012).

MA, YONGQING

Abstract: *Adaptability Evaluation of Panicum virgatum L. Cultivars on the Loess Plateau of China* (with Junfeng Shui, Yu An, Yongqing Ma, Nobumasa Ichizen, Shengpeng Zuo)

In the present study the growth traits and photosynthesis effect of several selected cultivars of switchgrass (*Panicum virgatum* L.) were determined. And then their cells morphology characteristics by microscopical anatomy and electron microscopic (EM) observation were further identified. It was expected to evaluate ecological adaptability of switchgrass to the Loess Plateau of China as a typically arid region. It was discovered that to a certain extent, the ecotype and chromosome ploidy would affect the co-evolution adaptability of switchgrass with the environment. It meant that it was unsuitable for Alamo and Kanlow of the lowland ecotypes to cultivate in the semi-arid area on the Loess Plateau of China. However, the upland cultivars with a high chromosome ploidy (e.g. 8n), for more efficient photosynthesis effect, would develop better than those of a low one. By EM a great deal of silica cells and vacuole-shaped ones as well as leaves trichome were found in switchgrass plants tissues like stem, leaf and root, which implied that switchgrass with the morphology can meet with arid habitat and dry climate. Therefore, if switchgrass would be introduced and extended on the Loess plateau of China, the cultivar of upland ecotype with a high chromosome ploidy would be the first and optimal choice.

Bio: Dr. Yongqing Ma is professor in the Department of Watershed Ecology, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources. He is a Founding Members of International Allelopathy Society and Chinese Allelopathy Society. Dr. Yongqong Ma has B.A.s . in Northwest Agricultural University, Shaanxi Province, P. R. China in agronomy in 1984. From July 1984 to January 1995 Dr. Yongqing Ma worked at Shijiazhuang Institute of Agricultural Modernization, Chinese Academy of Sciences, Shijiazhuang, P.R. China as Assistant Researcher and Associate professor, respectively. Dr. Yongqing Ma received a scholarship from Chinese Academy of Sciences and studied in the Department of Agronomy & Soil Science, University of New England, Armidale, 2351, N.S.W. Australia as a visiting scholar from October 1988 to December. 1989. From January. 2005 to March 1999 he received scholarship from Ministry of Education (Menbushu) and he received his Ph.D in Agricultural Resources from Arid Land Research Center, Tottori University, Japan in 1999. From August 1999 to August 2001 he received a scholarship from Japan Society of Promotion of Science as postdoctoral fellow at Arid Land Research Center, Tottori University, Japan working on search of *Striga* germination stimulant from non-host plant tissue cultures. From April 2005 to September 2005 Dr. Yongqing Ma was selected and worked as a visiting professor at Wild Plant Research Center, Utsunomiya University, Japan. From August 2001 to now Dr. Yongqing Ma worked at Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources as professor and Ph.D student supervisor. From September 2005, he was selected as Ph.D student supervisor at Northwest Sci. & Tech. University of Agriculture and Forestry, Shaanxi Province, P.R. China

Professor Ma's laboratory works on two aspects related to plant science: 1) Allelopathy in crop and pasture; and 2) Parasitism of root parasitic weeds (*Striga*, *Orobanche* and *Cistanche*). ; In allelopathy study Dr. Yongqing Ma mainly conducted research on winter wheat, *Cucurbita moschata*, *Astragalus adsurgens* Pall, *Stelleria chamaejasme* L. and published papers in Allelopathy Journal and some Chinese Journals.

Dr. Ma's another main research aspect is control of parasitic weeds of *Striga* and *Orobanche*. In this aspect he has worked on *Striga hermonthica* (Del.) Benth germination stimulant(s) from *Menispermum dauricum* (DC.) root culture and effect of medium composition on production of *Striga hermonthica* (Del.) Benth germination stimulant(s) by *Menispermum dauricum* (DC.) root cultures and published papers on Journal of Agricultural and Food Chemistry and Agronomy Journal. He also worked on germination induction of *Cistanche deserticola*, a traditional medicinal plant with the problem of germination, and he discovered a method for artificially induction of its germination. In

last year after completing a National Natural Science Foundation of China, he obtained a research project on “suicide germination” of *Orobanche* seeds by traditional medicinal plants. Dr. Ma also worked on pasture plant including switchgrass since 2005 and tried to select better adoptable varieties or by breeding an adoptable line in loess plateau of China. Dr. Ma’s work has been recognized on campus with awards in 2009 as the best teacher in the University and honors as one of the best researcher on innovation culture in Chinese Academy of Science in 2009.

MANN, DAVID

Abstract: *Switchgrass Biotechnology in the Bioenergy Science Center* (with Neal Stewart)

The Bioenergy Science Center (BESC) is one of three bioenergy centers created by the US DOE in 2007, and is focused on decreasing the recalcitrance of cellulosic feedstock for conversion into fuel. Biotechnology of switchgrass is important in screening potential cell wall biosynthesis genes, which in BESC is being performed by five laboratories in three institutions with additional laboratories contributing key technologies. Accomplishments include altering lignin biosynthesis, improved tissue culture and transformation systems, a protoplast system, and a new vector set for monocot transformation. A transgene pipeline committee identifies transgenes to be evaluated (overexpression or knockdown) in switchgrass. Stable transformants are produced to evaluate gene function of interesting transgenes. One example is in the lignin biosynthesis pathway, in which we have produced stable transformants that are currently being evaluated. In order to coordinate gene expression and to facilitate more rapid screening of genes, we have developed a Gateway-compatible monocot transformation vector set for overexpression (high or low), RNAi, visible and selectable markers. BESC has facilitated coordination of expertise and duties to perform research in switchgrass biotechnology that would have been impossible in any individual laboratory.

Bio: David Mann graduated with a Bachelor of Science in Biology from Bryan College in 2002. After internships at the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) and Oak Ridge National Laboratory in 2002 and 2003, he received his Ph.D. in Microbiology working under Gary Saylor at the Center for Environmental Biotechnology at the University of Tennessee in 2008. His dissertational work focused on the application of nanotechnology to biology, and involved the development of novel transformation methods. Since then, David has been working as a post-doctoral research associate in Neal Stewart’s laboratory, acting as lead on the lab’s work with the BioEnergy Science Center in the area of switchgrass biotechnology and transformation.

MEILAN, RICHARD

Abstract: *Genetically Engineering Poplars for Modified Lignin Composition in order to Improve Its Utility as a Bioenergy Feedstock*

High fuel prices, concerns about global climate change, and the limitations of international petroleum reserves have revived worldwide interest in renewable resources as feedstocks for liquid transportation fuels, especially those derived from cellulose. As a perennial woody plant, hybrid poplars (species within the genus *Populus*) offer several advantages as a bioenergy crop, including rapid growth rates, the ability to cycle nutrients, a wide geographic distribution, genetic diversity, amenability to genetic engineering, abundant genomic resources, greater flexibility with regard to harvest, and more efficient transport and storage. The phenolic cell-wall polymer lignin constitutes a significant barrier to biomass conversion for all crops that serve as cellulosic feedstocks; however, it is also essential to normal plant growth and development. Recent advances in our understanding of how lignin is synthesized provide us with an opportunity to modify the composition of the lignin polymer, in an effort to improve the efficiency of the cell-wall deconstruction phase during ethanol production. We have produced several hundred transgenic poplar lines containing constructs designed to modify lignin in various ways (both content and composition), all of which are in the process of being field-tested. Once the optimum lignin composition has been determined in transgenic trees, a

metabolic fingerprint will be developed to aid in the identification of individuals from conventionally bred and wild populations that possess similar wood properties. The results from this project may help to inform work being done with other bioenergy crops that are also expected to be used as cellulosic feedstocks. The outcome of this research will allow for the rational assessment of the cost savings that could result from using poplar, instead of maize (*Zea mays*), as a feedstock for producing biofuels.

Bio: Rick Meilan received a joint Ph.D. in Botany (emphasis in plant molecular biology) and Forestry (emphasis in tree physiology) from Iowa State University in 1990. After working as a Rockefeller Foundation-funded post-doctoral research fellow, he joined the faculty in Department of Biochemistry at the University of Missouri-Columbia. He was on the faculty in the Forest Science Department at Oregon State University for nine years before going to Purdue University (PU) in October 2003. At PU, he is an Associate Professor of Molecular Tree Physiology.

In his laboratory at Purdue, Meilan uses molecular tools to investigate the genetic mechanisms by which key aspects of tree growth and development are controlled. He is also attempting to domesticate and add value to various tree species by genetically engineering them to express genes that impart environmentally beneficial and commercially important traits. Active research projects include efforts to insert into poplar genes that affect lignin composition, salt tolerance, water-use efficiency, and poplar's ability to remediate contaminated sites. In addition, he and his group are trying to identify genes that regulate the transition from sapwood to heartwood in hardwoods, the formation of figured wood, and the initiation of lateral and adventitious roots. They are also trying to identify metabolites that influence the susceptibility of ash trees to an invasive pest, the Emerald Ash Borer.

NEBENFÜHR, ANDREAS

Abstract: *The Power of Movement in Plant Cells: Myosin Motors and Cytoplasmic Streaming* (Andreas Nebenführ, Eunsook Park, Jian-Feng Li, Stephanie Madison, Krzysztof Bobik)—Plant cells display a high degree of intracellular movements, often referred to as “cytoplasmic streaming.” Although the earliest description of this process dates back more than 200 years, we still have only rudimentary knowledge of the physiological function of these seemingly random motions during plant growth and development. To bring light into this question, we are studying the myosin motor proteins that are responsible for the movements by genetic, cell biological, and biochemical approaches. The model species *Arabidopsis thaliana* contains thirteen genes encoding myosin XI proteins which are believed to be responsible for organelle movement. We have isolated T-DNA insertion mutants for all genes in the family and found no or only mild phenotypes. However, two closely related paralogs were found to be involved in root hair elongation as the double mutant resulted in significantly shorter root hairs than wild-type. This phenotype may be related to the reduced accumulation of secretory vesicles at the tip of mutant root hairs. Myosins binding to organelles is mediated by its C-terminal globular domain. Homology modeling suggested that this domain of plant class XI myosins can assume a similar conformation as the corresponding region of animal and fungal class V myosin. Experiments of the organelle binding ability of various myosin truncation constructs in living plant cells revealed that the globular domain contains at least two separable binding sites and that organelle attachment is synergistically regulated with motor protein dimerization. These results suggest several levels of regulation that determine whether a given organelle will move actively through the cytoplasm.

Bio: Dr. Nebenführ is a native of Germany where he obtained his M.S. degree in Molecular Biology and Genetics from the University of Freiburg. He then moved to the US and received his Ph.D. in Molecular and Cellular Biology from Oregon State University, working on molecular responses to the plant hormone auxin. He received postdoctoral training at the University of Colorado in Boulder in the area of the secretory apparatus of plant cells. Since 2001, Dr. Nebenführ is on the faculty of the

University of Tennessee in Knoxville where his lab studies the mechanisms of fast intracellular movements during cytoplasmic streaming.

SAYLER, GARY

Bio: Dr. Sayler is the Beaman Distinguished Professor in the Department of Microbiology, Ecology and Evolutionary Biology at the University of Tennessee, Knoxville; director of the University of Tennessee-Oak Ridge National Laboratory Joint Institute for Biological Sciences; adjunct professor at Gwangju Institute for Science and Technology, South Korea; and Honorary Professor at East China University, Shanghai. He is the founding Director (1986) of the University of Tennessee Center for Environmental Biotechnology. His research interests include microbiology, toxicology, and molecular biology of biodegradation, of toxic pollutants such as PCB and PAH. He pioneered the development of environmental molecular diagnostics including the extraction and analysis of nucleic acids from the environment and wastes, environmental gene probe analysis, bioluminescent bioreporter/sensor technology, and conducted the first field release of a genetically-engineered microorganism for remediation process monitoring and control. Over his career, Dr. Sayler has built and directed programs of approximately \$100 million in environmental research, edited five books, and contributed over 300 publications and 500 invited presentations. He holds 16 patents on environmental gene probing, genetic engineering for bioremediation, biosensor technology, and environmental gene expression. He received the NIEHS' Research Career Development Award (1980-1985); was named a Top 100 Innovator in Science by Science Digest (1985); received the American Society for Microbiology, Procter and Gamble Award for Environmental Microbiology (1994), the Distinguished Alumni Award of the University of Idaho (1995), the DOW Chemical Foundation SPHERE Award (1998-2000), as well as the Chancellor's Research Scholar Award and the Arts and Sciences Senior Researcher award from the University of Tennessee. He was elected to the American Academy of Microbiology in 1991. Dr. Sayler has served on numerous of councils and committees for the National Research Council, DOE, EPA, NIEHS, NSF, NASA, and WERF. He is currently a member of the U.S. Environmental Protection Agency's Science Advisory Board, Chair of the Board of Scientific Counselors for EPA's Office of Research and Development, and is a member of the DOE/OS Biological and Environmental Research Advisory Committee. Dr. Sayler is a member of five professional societies and currently serves on five editorial boards and is associate editor of *Environmental Science and Technology*. Dr. Sayler has directed graduate research of approximately 50 doctoral and 15 master's students in microbiology, ecology and evolutionary biology and engineering.

SHEN, SHI-HUA

Abstract: *The JcDof1 transcription factor, a new clock protein, is associated with the photoresponses in Jatropha curcas* (Jing Yang, Mingfeng Yang, and Shihua Shen)—*Jatropha curcas* is drawing the attention of the world as a biodiesel plant in recent years. Understanding the function of genes responsible for the regulation of growth and development is a prerequisite for breeding energy plants with high adaptability to a wide range of environment conditions, for example, various light cycles. From *J. curcas* seedlings, a new member of DOF family was isolated, and hence the name JcDof1. A conserved DOF-DNA-binding domain was demonstrated in the deduced amino acid sequence of JcDof1. Further investigation indicated that the JcDof1 was localized in the nucleus, and exhibited abilities of DNA binding and transcriptional activation. The expression of the JcDof1 gene changed rhythmically under long day, short day, and constant light conditions. Taken together, the results showed that JcDof1 functions as a novel transcription factor involved in the circadian clock of *J. curcas*, and its function would help us to understand better the complex nature of the circadian clock in woody oil plants.

Bio: Prof. Shihua Shen is a research scientist in Institute of Botany, the Chinese Academy of Sciences. One of his main interests is research on the molecular mechanisms of plant responses to stress environmental signals using rice as a model plant through new high throughput methods such as proteomics, with an emphasis on drought, low temperature, and salinity; another is to exploit high energy-store varieties that can be cultivated on marginal land using genetic engineering. Current research is the regulation of oil metabolism, stress related genes, and the function of transcription factors responsible for growth, flowering, etc. in *Jatropha curcas*. The goal of this project is to discover the important genes and proteins for regulation of energy enrichment, stress tolerance and growth in this promising biodiesel plant, to apply molecular design to energy plants, and finally to provide renewable fuel while protecting natural resources.

SHUI, JUN-FENG

Abstract: *Allelopathic effect of Panicum virgatum L. on alfalfa (Medicago sativa L.) and perennial ryegrass (Lolium perenne L.) and its chemical basis* (Junfeng Shui, Yongqing Ma, Nobumasa Ichizen, Shengpeng Zuo)—In the present study, allelopathic effect of switchgrass (*Panicum virgatum L.*) on alfalfa (*Medicago sativa L.*) and perennial ryegrass (*Lolium perenne L.*) as well as chemical basis was conducted. Switchgrass showed the most significant inhibitory effect on receptor species only under the treatment of tested original extracts (1:10 g/ml). When the first extracts were diluted, allelopathic inhibition from switchgrass would decline distinctly and even disappear at the end. It was found that allelopathic potential of switchgrass was correlated closely with its ecotype, but not with its chromosome ploidy. The plants grown in the upland displayed stronger allelopathic potential than that of lowland condition. The growth of radicles and hypocotyls of receptor materials was more sensitive to aqueous extracts of switchgrass than that of the germinating seeds. Especially, allelopathic inhibition from aboveground agents of switchgrass was more evident than that of roots tissues. Based on the above facts, the inner allelochemicals in switchgrass were isolated preliminarily and simply bio-assayed. There existed four kinds of dominant compounds such as phenols, organic acids, neutral chemicals and alkaloids in switchgrass. Among them, the alkaloids in switchgrass markedly suppressed the seedlings growth of perennial ryegrass, and the inhibition of seeds germination and seedling development of alfalfa mainly caused by organic acids. It implied that different allelochemicals types exhibited the selective inhibitive effect on target receptors.

Bio: Dr. Junfeng Shui is an assistant professor in the Department of Watershed Ecology, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources. He has B.E.s. in Northwest University, Xi' An, Shaanxi Province, P. R. China in bioengineering in 1997. From July 1997 to present, he works at Institute of Soil and Water Conservation, CAS. He is a Ph.D student in Ecology of CAS. Dr. Shui works in Professor Yongqing Ma's laboratory as an assistant, and his work relates to plant science: allelopathy in crop and grass. In allelopathy study Dr. Shui mainly conducted research on cucumber and switchgrass (*Panicum virgatum L.*) and published papers in Allelopathy Journal and Chinese Journals.

STEWART, NEAL—Workshop Chair

Bio: Dr. Neal Stewart holds the Racheff Chair of Excellence in plant molecular genetics and is a professor in the Department of Plant Sciences at the University of Tennessee. He has degrees from North Carolina State University (B.S.) and Virginia Tech (M.S. and Ph.D) with postdoctoral experience at the University of Georgia. Dr. Stewart's research spans plant biotechnology, genomics, and ecology. Much of the work has been with the production and field research of transgenic plants, especially with regards to their biosafety. Recently, his research program has entered the field of bioenergy, especially in the biotechnology, genetics, and systems biology of switchgrass as a transportation fuel feedstock. To that end in the past half year, there has been a total of over \$2.1

million USD invested in his switchgrass research program from the U.S. Department of Energy, the Southeastern SunGrant Center and from a private company. He is a key person in the ORNL's Bioenergy Science Center. One goal of this research is the environmentally sustainable and responsible deployment of biotechnology solutions for switchgrass improvement for bioenergy feedstocks. Dr. Stewart is author of over 150 publications and has most frequently published works in Nature Biotechnology. Over the past 10 years he has been the principle investigator of over \$8 million USD in grants and has 9 issued or pending patents. He is the co-founder and Chief Technology officer of a startup biotechnology company (Mycogenomix), which is focused on insect control for agricultural plants.

TUSKAN, GERALD

Abstract: *Resequencing, Snp Discovery, and Association Genetics in Populus* (with Gancho Slavov, West Virginia University; Tongming Yin, ORNL; Xiaohan Yang, ORNL; Lee Gunter, ORNL; Ranjan Priya, ORNL; Xinye Zhang, ORNL; and Steve DiFazio, West Virginia University)—As one of the fastest growing woody perennial plants, one which can be easily clonally propagated through the use of unrooted lateral branches, *Populus* provides fiber for pulp, paper, veneer, and bioenergy feedstocks. The domestication process however has been erratic and slow due to the large size and delayed reproductive habit of this woody perennial tree. In an effort to identify genes controlling economically important phenotypes, modern genetics and genomics tools and approaches are being brought to bear on the efforts to accelerate the domestication process in *Populus*. Twenty alternate *Populus* genotypes have been resequenced and aligned to the reference genotype Nisqually-1 in order to identify single nucleotide polymorphisms across the entire *Populus* genome. Using the Solexa 72-bp paired-end platform, and a 30X depth for each alternate genotype, we have identified ca. 14 million single nucleotide polymorphisms (SNP) uniformly distributed across the genome. SNP discovery was affected by the reference genome, genome position, intragenomic duplications and assembly algorithms. Within an 1100-genotype association study, linkage disequilibrium decays within 100 bp. Using a combining quantitative trait locus analyses and association genetics approaches we have identified candidate genes related to drought tolerance, crown architecture, cell wall chemistry and disease resistance in *Populus trichocarpa*. Utilizing this information has made it possible to refine genetic maps, improve candidate gene characterization, and test hypotheses related to accelerated domestication.

Bio: Dr. Gerald Tuskan is a distinguished scientist in Environmental Science Division (ESD) at ORNL. His major research interests are plant genomics, such as genetic basis of tree growth and development with emphasis on biomass accumulations, carbon allocation and cell wall chemistry, *Populus* genomics - assembly of the draft sequence, comparative genomics and functional gene identification, and short-rotation woody crop silvicultural systems. Dr. Tuskan received his Ph.D. in Genetics from Texas A&M University in 1984, a master's degree in Forest Genetics from Mississippi State University in 1980, and a bachelor's degree in Forest Management from Northern Arizona University in 1978.

XU, BING-CHENG

Abstract: *Seedling biomass partition and water use efficiency of switchgrass and milkvetch under monoculture and mixture in response to various water availabilities* (Bing-Cheng Xu, Xi-Ping Deng, Sui-Qi Zhang, and Lun Shan)—Seedling biomass production and allocation, transpiration water use efficiency (TWUE), and species competition of switchgrass and milkvetch were investigated in a pot-cultivated experiment under different levels of water availability conditions. The experiment was conducted using a simple replacement design in which switchgrass and milkvetch were grown in a greenhouse at an overall density of ten individuals per pot, providing combinations of the two species as 0:10, 5:5 and 10:0. Five water levels which were sufficient water supply (HW),

soil drying gradually from HW (DHW), moderate water stress (LW), soil drying gradually from LW (DLW), and rewatering back to LW after short period of drying from LW (RLW) over a 15-day period were applied. Biomass production accumulation and its partitioning, and transpiration water-use efficiency (TWUE) were recorded at the end of the experiment. Species competitive indices such as competitive ratio (CR), aggressivity (A) and relative yield total (RYT) were calculated from the biomass dry weight data for shoot, root and total, respectively. Water stress significantly reduced seedling biomass production while increased root: shoot ratio under monoculture or mixture. Only switchgrass showed compensatory in biomass and TWUE under RLW when mono-cultured, while both species demonstrated compensatory growth under RLW in the mixture. Switchgrass was the dominant species and much more aggressive than milkvetch under LW treatment, while in the other four treatments milkvetch was the dominant species as measured by the positive value of aggressivity and higher values of CR. The total biomass RYT values of the two species were higher than 1.0, indicating some degree of resource complementarity. Although the biomass production was lower than sole milkvetch, the two species combination had advantage in TWUE especially under low and fluctuating water available conditions.

Bio: Dr. Bingcheng Xu is an associate professor in State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences (CAS) and Ministry of Water Resources (MWR). He got his BA in soil and water conservation from Northwest Forestry University (now The College of Forestry, Northwest A & F University) in Yangling, Shaanxi Province, P. R. China in 1996. From September 1996 to July 1999, Dr. Bingcheng Xu studied at the Graduate School of the Chinese Academy of Sciences in Beijing and Institute of Soil and Water Conservation, CAS and MWR, and got his MS in Ecology in 1999. In 2003, he received his Ph.D. in Soil Science from Northwest A & F University. Dr. Bingcheng Xu joined the Institute of Soil and Water Conservation, CAS, and MWR in July 1999, and then worked as an assistant researcher and associate professor, respectively.

XU, ZENG-FU

Abstract: *Analysis of Expressed Sequence Tags from Biodiesel Plant *Jatropha curcas* Embryos at Different Developmental Stages* (Mao-Sheng Chen, Gui-Juan Wang, Ru-Ling Wang, Jun Wang, Song-Quan Song, and Zeng-Fu Xu)—*Jatropha curcas*, a tropical and subtropical shrub of the family Euphorbiaceae, is considered a potential biodiesel feedstock plant whose seeds contain up to 40% oil. However, little is currently known about the molecular biology of *J. curcas* seeds. Therefore, it would be valuable to understand the mechanisms of development and lipid metabolism in *J. curcas* seeds. This understanding would be helpful in using genetic engineering to develop new *J. curcas* cultivars. In this study, three cDNA libraries were constructed from *J. curcas* embryos at different stages of seed development. A total of 9844 expressed sequence tags (ESTs) were produced from these libraries, from which 1070 contigs and 3595 singletons were obtained. Over 35% of these unigenes did not have a significant match with sequences in the present databases, suggesting that these sequences represent novel genes or non-coding RNAs. One hundred and seven unigenes were found to be differentially expressed at the three stages of seed development, indicating that these genes may play key roles in seed development of *J. curcas*. In addition, 59 and 61 unigenes have been identified whose putative function may be involved in development and lipid metabolism in *J. curcas* seeds, respectively. Some of these genes also play important roles in embryogenesis, morphogenesis, defense response and adaptive mechanisms in plants. To our knowledge, this is the first EST library publicly deposited for *J. curcas*, which can provide valuable resource for understanding the developmental and metabolic processes of *J. curcas* seeds, the cloning of new genes, annotation of genomic sequence and the development of molecular marker for gene mapping, polymorphism and marker-assisted selection breeding of *J. curcas* in the future. Furthermore, to further investigate the developmental and metabolic mechanisms of *J. curcas* seeds, endosperm cDNA libraries should be constructed to identify genes related to these processes.

Bio: Dr. Zeng-Fu Xu is a principal investigator and head of the Laboratory of Molecular Breeding of Energy Plants, Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences (CAS), China, and a Professor of Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Sun Yat-sen University, Guangzhou, China. The major research areas Dr. Xu has been focused on are gene expression, physiological function, agricultural and medical applications of plant proteases and their inhibitors. More recently, his research interest has also been extended to the functional genomics and genetic engineering of the biodiesel feedstock plant *Jatropha curcas*, a tropical and subtropical shrub of the family Euphorbiaceae.

Dr. Xu now serves as the coordinator of the Energy Plant Research Consortium (EPRC) at XTBG. The main objective of the EPRC at XTBG is to improve seed yield and oil content of *Jatropha* through molecular breeding approaches combined with traditional breeding procedures, and to develop *Jatropha* as a model plant for biodiesel feedstock research. Functional genomics and genetic engineering approaches are used to understand the growth and development of *Jatropha* at the molecular level, and to obtain transgenic *Jatropha* plants with increased seed and oil yield and with enhanced tolerance to abiotic and biotic stress. *In vitro* (tissue culture) propagation techniques of elite *Jatropha* plants are explored to produce high quality seedlings for large scale *Jatropha* plantation.

YANG, XIAOHAN

Abstract: *Quantitative analysis of Populus gene expression in response to drought stress using next-generation sequencing technology* (Xiaohan Yang, Timothy J. Tschaplinski, Sara Jawdy, Lee E. Gunter, Gerald A. Tuskan and Christa Pennacchio)—*Populus* (poplar) is a potential biomass feedstock for biofuel production. Global warming will produce an increased probability of episodic drought, potentially causing severe problems in biomass production. Understanding of poplar gene expression in response to drought stress is a prerequisite for creating cis-genic poplar plants with enhanced drought tolerance, which can be grown on marginal lands for biofuel production. Traditional cloning-based EST sequencing and microarray, which have been used for poplar gene expression studies, suffer several limitations. For example, traditional EST library sequencing generally misses a significant portion of transcripts. Current poplar microarrays are largely dependent on genome annotation, and some of the microarray probes are not highly specific. To avoid these limitations, we performed global profiling of *Populus* gene expression in response to drought stress using the 454 sequencing technology. We identified a set of more than 500 genes which were up-regulated by dehydration. The gene ontology analysis revealed that the genes relevant to drought response were enriched in this gene set. Our research demonstrated that sequencing-based profiling is an excellent approach for the quantitative analysis of *Populus* gene expression.

Bio: Xiaohan Yang is a staff scientist of Oak Ridge National Laboratory. His main interest is in computational functional genomics at the genome scale in the evolutionary context. He is currently an active member in the DOE BioEnergy Science Center (BESC) responsible for gene family analysis, construct design, *in silico* identification of genes associated with cell wall biosyntheses, and analysis of *Populus* transcriptome sequencing data. Besides his BESC activities, he is working on a DOE-funded project to profile the leaf transcriptome of *Populus* under drought stress using new high throughput sequencing platforms. Also, he is involved in the development of high-performance computing capabilities for genome analysis.

ZHUANG, JIE—Workshop Coordinator

Bio: Dr. Jie (Joe) Zhuang is a research director for the Institute for a Secure and Sustainable Environment (ISSE) and a research associate professor in the Department of Biosystems Engineering and Soil Science at the University of Tennessee. He is also the coordinator of the China-U.S. Joint Research Center for Ecosystem and Environmental Change. Over the past two decades, Dr. Zhuang

has worked on many challenging scientific research projects in the United States, Japan, and China. His research is focused on the fate and transport of contaminants (viruses, radionuclides, colloids, and munitions constituents) in the environment; soil carbon management; soil hydrology; and carbon-water-nitrogen fluxes of terrestrial ecosystems. He has published more than 40 research papers on high-profile international journals. Dr. Zhuang was a research fellow of Japan Society for Promotion of Science from 1998 to 2000. Currently, he is an editorial board member for three international journals, *Ecotoxicology*, *Environmental Management*, and *Pedosphere*. Dr. Zhuang also contributes a significant amount of his time to the China-U.S. Initiative launched by the University of Tennessee/Oak Ridge National Laboratory's Joint Institute of Biological Science and ISSE, with the aim of promoting U.S.-China collaborations in the areas of global environmental change, bioenergy sustainability, and international education.

POSTER PRESENTATIONS

BLAKE, JOYCE

Introducing the 'diesel trees' (Copaifera spp.) as an alternative source of novel chemicals for biofuel (Blake L. Joyce¹; Feng Chen¹, Hani Al-Ahmad^{1,2}, Bruce Bunting³, John M. Storey³, Nan Zhao¹, Tobias Köllner⁴, Jörg Degenhardt⁴, C. Neal Stewart¹ - ¹Department of Plant Sciences, University of Tennessee, Knoxville, TN; ²Department of Biology and Biotechnology, An-Najah National University, Nablus, Palestine; ³Fuels, Engines and Emissions Research Center, Oak Ridge National Laboratory, Oak Ridge, TN; ⁴Max Planck Institute for Chemical Ecology, Beutenberg Campus, Jena, Germany)

Abstract: When trees in the genus *Copaifera* are tapped they produce oleoresins which are rich in sesquiterpenes (80-90%) and diterpenes (5-10%). These oleoresins are used in cosmetics, the food industry, as herbal medicine in South America, and as fuel to power diesel engines. Melvin Calvin originally explored the properties of these trees in the 1980s as a potential source of plant-derived hydrocarbons, but since this time there has been little formal investigation into their diesel-like properties. Traditional forestry practices for the production of the oleoresin seems unfeasible for the biodiesel market due to the long generation time, low yields, and the tropical nature of the trees that limits their range. Despite this fact, these oleoresins represent an interesting chemistry new to biodiesels as they are comprised mostly of cyclic hydrocarbons (primarily β -caryophyllene) from the isoprenoid pathway. While the chemical constituents of the oleoresins are well documented, the biochemical pathway and molecular biology of these plants has not yet been studied. More understanding of the *Copaifera* isoprenoid synthesis pathway could lead to use of these genes in temperate oilseed crops to improve their suitability for use as biodiesel. We have identified the presence of the sesquiterpenes of interest in root, stem, and leaf tissues of seedlings and two year old *Copaifera officinalis* trees. Additionally, based on cluster analysis of the 26 sesquiterpenes detected we predict that at least four terpene synthases (TPS) are involved in the sesquiterpene biosynthesis pathway. Further scientific investigation into the oleoresin fuel properties is needed to determine whether the oleoresins will function as biodiesel in modern engines and their potential use in blending with other biodiesels.

BURRIS, JASON

Novel switchgrass transformation procedure

Abstract: The utilization of switchgrass (*Panicum virgatum*) for use as a biofuel is being explored. Current protocols for the transformation of switchgrass require months of transformation/regeneration and provide relatively poor transformation efficiencies. Here we have demonstrated a tissue culture system, utilizing a novel media, LP9, that demonstrates decreased time to the production of whole transgenic plants and with increased efficiency. Our system is optimized for the production of Type II callus which has demonstrated higher transformation efficiencies in other monocots. By selecting only Type II callus to be used in the transformation process, we aim to increase transformation efficiency. Currently, we have increased the transformation of switchgrass to up to 4%.

HARPER, DAVID

Characterization of lignins using thermal and FT-IR spectroscopic analysis (R. J. Sammons¹, Nicole Labbé¹, David P. Harper¹, Thomas Elder², and T. G. Rials¹ [¹Forest Products Center, The University of Tennessee, 2506 Jacob Drive, Knoxville, TN 37996, ² Southern Research Station, USDA Forest Service, 2500 Shreveport Highway, Pineville, LA 71360])

Abstract: A selected group of biomass derived lignins isolated using clean fractionation, an Organosolv process, was characterized using FT-IR spectral and thermal property analysis coupled with multivariate analysis. PCA indicated that there were significant variations between the hardwood, softwood, and grass lignins due to the difference in syringyl and guaiacyl units as well as the processing temperature and time used to extract the lignins. The PLS revealed that the concentration of syringyl units is the foremost factor in the variation of glass transition temperature (T_g) for each lignin sample. It was concluded that the structural variations resulting from altering the processing time and temperature and the lignin species, directly affect the thermal properties of the lignin. Therefore, by determining the thermal properties of a lignin sample, a basic understanding of its structure can be developed.

HAYES, DOUGLAS G.

Chemical Analysis of Biomass Facilitated by Dissolution into Ionic Liquids (Lindsey M. Kline^{a,b}, Charles Warren Edmunds^a, Douglas G. Hayes^a, Alvin R. Womac^a, and Nicole Labbe^b -- ^aDepartment of Biosystems Engineering & Soil Science; ^bTennessee Forest Product Center, University of Tennessee, Knoxville, TN 37996)

Abstract: A new simple and rapid method for quantifying lignin content in lignocellulosic biomass, based on the spectrophotometric analysis of whole biomass dissolved in ionic liquids, is described. The approach consists of measuring the absorbance of a solution of biomass dissolved in the ionic liquid 1-n-butyl-3-methyl imidazolium chloride, [Bmim][Cl], at 440 nm via ultraviolet-(UV-) visible spectrophotometry. An extinction coefficient for a lignin standard, highly pure lignin isolated from biomass through an organosolv process, is used in conjunction with the Beer-Lambert Law to calculate the lignin concentration. Principal component analysis (PCA) of Fourier Transform-Infrared (FTIR) spectra collected for several different lignin standards was performed to understand the differences in their chemical structure and composition (e.g., the relative amounts of syringyl and guaiacyl units), and to assist in the proper selection of the standard for the spectrophotometric analysis. The spectrophotometric approach yielded lignin contents for Yellow poplar and Southern pine of $25.7 \pm 1.1\%$ and $26.7 \pm 0.7\%$, respectively, which are comparable to the values obtained by a standard wet chemical protocol, $25.1\% \pm 0.7$ and $26.6 \pm 0.4\%$, respectively. The dissolution of biomass into ionic liquid has been readily monitored through use of light microscopy, with digital images processed using a Matlab computer program prepared by undergraduate researchers.

JIANG, ZHE

Biobased polyesters and composites using 2-ketoglutaric acid and aliphatic alcohols from sustainable resources (Zhe; Jiang¹, Joseph J. Bozell¹, Mark D. Dadmun² -- ¹Forest Products Center-Biomass Chemistry Laboratories, University of Tennessee, Knoxville, TN 37996; ²Department of Chemistry, University of Tennessee, Knoxville, TN 37996)

Abstract: Novel biobased materials have attracted increasing interest in recent years. Biobased materials from renewable resources not only hold a variety of advantages including carbon-neutrality and biodegradability over their petrochemical counterparts, but offer many new properties and applications unavailable in the past. Certain cost competitive materials including Natureworks' PLA, Dupont's Sorona and Bionolle have been commercialized. We are investigating 2-ketoglutaric acid (2-KGA), an intermediate from the well-known citric acid cycle and a potential biobased building block available from the enzymatic conversion of biorefinery sugars, for its reactivity with various biobased aliphatic diols in polycondensation processes to produce polyesters. In this work, the results of various reactions attempting to synthesize such polyesters will be discussed, as will the possibilities of producing cellulose-polyester composite materials.

MANN, DAVID

*Baseline biomass characterization and comparison of switchgrass (*Panicum virgatum* L.) grown under various conditions*

Abstract: Switchgrass (*Panicum virgatum* L.) is a warm-season perennial grass native to North America. Because of various traits naturally possessed by switchgrass, it has been targeted as a lignocellulosic feedstock for bioenergy. However, the complexity of the cell wall and recalcitrance attributable to compositional elements such as lignin currently renders the utilization of switchgrass for ethanol production economically unfeasible. Therefore, strategies are underway to genetically engineer switchgrass with modified cell walls that are more easily degradable for saccharification and fermentation. In order to study the alterations of cell wall components, baseline data of switchgrass biomass composition are needed. In this study, two switchgrass genotypes (Alamo and Alamo 2) were grown under different environmental conditions and harvested at the reproductive growth stage. Switchgrass plants were divided into separate components (flower, leaf, node/internode) and analyzed for compositional variation using molecular beam mass spectrometry (MBMS) and wet chemistry.

Isolation and Expression of a Switchgrass Polyubiquitin Promoter for Overexpression in Monocot and Dicot Transformation Systems

Abstract: Switchgrass (*Panicum virgatum* L.) is a promising feedstock for the production of cellulosic ethanol, and switchgrass biotechnology plays a pivotal role in the BioEnergy Science Center initiative. Desirable phenotypes for the reduction of recalcitrance will be achieved by overexpressing or down-regulating genes of interest using a strong constitutive promoter. Currently, the number of promoters demonstrated to regulate strong expression in switchgrass is very limited, and more are needed. We have identified an active promoter region upstream of an ubiquitin coding region from a genomic library of switchgrass (*Panicum virgatum*). This region at the 5' end of the ubiquitin gene contains a promoter and a putative intron, termed PvUbi1, regulates strong constitutive expression in a number of switchgrass tissue types, and is capable of driving transgene expression in switchgrass (*Panicum virgatum*), tobacco (*Nicotiana tabacum* Xanthi) and a fern species (*Pteris vittata*). In addition, in-frame fusions of the first three and nine amino acids of the ubiquitin gene to the 3' end of the promoter and intron also regulated increased levels of transient expression of GUS in these plant species. Based on these data, PvUbi1 will be a valuable promoter candidate for monocot, dicot and fern transformation systems.

MAZAREI, MITRA

Switchgrass cell suspension cultures: establishment, characterization, and application (M. Mazarei¹, H. Al-Ahmad^{1,2}, M. R. Rudis¹, B. L. Joyce¹, and C. N. Stewart, Jr.¹ [¹Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996, USA; ²Department of Biology and Biotechnology, An-Najah National University, Nablus, Palestine])

Abstract: Switchgrass (*Panicum virgatum* L.) is a warm-season perennial grass that is native to the prairies of North America. It is grown in monoculture for hay, grazing, erosion, and is a potential source for biofuel production as a feedstock. Genetic manipulation of switchgrass is needed for better cellulosic ethanol production, especially to improve cellulose-to-lignin ratios. Cell suspension cultures offer an in vitro system for mutant selection, mass propagation, and gene transfer experiments. The objective of this study was to produce a useful cell suspension system for such applications as plant regeneration, protoplast isolation, and cell biology in view of cell wall traits assessment. Switchgrass cell suspension cultures were initiated from embryogenic callus obtained from the inflorescences of genotype Alamo 2. They were established as different cell types and named sandy, fine milky, and ultrafine cultures. Histological analysis using scanning electron microscopy signified morphological distinction between the cell suspension type cultures. As an initial evaluation, each cell type was examined for protoplast isolation efficiency. A high protoplast

isolation rate of up to 106 protoplasts/1.0 g of cells was achieved for the fine milky culture, whereas only a few protoplasts were isolated for the sandy and ultrafine cultures. These results indicate that switchgrass cell suspension type sizably impacts the efficiency of protoplast isolation, pointing out its possible significance in other applications.

MOON, HS

Biocontainment using pollen-active recombination systems (Hong S. Moon¹, Wei Wei^{1,2}, Yi Li³, and C. Neal Stewart, Jr.¹ -- ¹Department of Plant Sciences, University of Tennessee, Knoxville, TN 37923; ²Institute of Botany, Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Beijing 100093, PR China; ³Department of Plant Science, University of Connecticut, Storrs CT 06269)

Abstract: Switchgrass (*Panicum virgatum* L.), a perennial warm-season forage plant that is native to the US, is one of the leading potential sources for biomass-based renewable energy. Switchgrass is being genetically modified to increase cellulosic ethanol yields and will likely be commercially cultivated under large-scale agronomic conditions. Large-scale farming of transgenic switchgrass could trigger transgene escape from transgenic switchgrass populations to wild populations. Since there have been no gene flow studies for switchgrass, regulators will likely be concerned about transgene escape from transgenic switchgrass. Switchgrass is known as a wind-pollinated obligate-outcrosser, thus biocontainment of transgenes from transgenic switchgrass would be desirable to eliminate, at least decrease, transgene spread via pollen. Transgene excision from pollen using a site-specific recombination system could be an effective tool to suppress unwanted transgene flow. All functional transgenes will be flanked by the corresponding recognition sites the recombinase. If this transgene excision system is successful in switchgrass, it may be practically employed to other monocotyledonous crops in agronomic field conditions.

O'LENICK, C. J.

Solvent fractionation of switchgrass: Pretreatment for lignocellulosic bioethanol production (C.J. O'Lenick¹, Joseph J. Bozell¹, Timothy J. Rials², Anton Astner³, Rene Buchinger³, Stuart Black⁴ -- ¹Forest Products Center - Biomass Chemistry Laboratories, University of Tennessee, 2506 Jacob Drive, Knoxville, TN 37996; ²Director of R&D, Office of Bioenergy Programs, University of Tennessee, 2506 Jacob Drive, Knoxville, TN 37996-4570; ³University of Applied Sciences Salzburg, Campus Kuchl, Markt 136a, A-5431 Kuchl, Austria; ⁴National Bioenergy Center, National Renewable Energy Laboratory, 1617 Cole Blvd, Golden, CO 80401)

Abstract: Interest in recovering cellulose from biomass has grown steadily over the last few years as cellulose can be enzymatically hydrolyzed to sugars and fermented to ethanol. Under the Energy Independence and Security Act of 2007, refiners must produce 16 billion gallons of cellulosic ethanol per year by the year 2022. Switchgrass is a promising lignocellulosic source for this ethanol. Switchgrass is a perennial non-food plant, which can grow in different weather conditions and in poor soils that cannot support food crops. We are investigating solvent fractionation to isolate the required cellulose from switchgrass to make biofuel. Solvent fractionation is a process of choice for pretreatment, because it is suitable for use with several different biomass feedstocks, giving (i) favorable separations, (ii) easy isolation of products after fractionation, and (iii) recovery of each component in a high yield and purity amenable to conversion to other chemicals. This technology, originally developed at the National Renewable Energy Laboratory, is being used at The University of Tennessee for the separation of switchgrass into its primary components. The fractionation employs a ternary mixture of methylisobutylketone, ethanol and water to separate switchgrass into cellulose, lignin and hemicellulose for the production of fuels and chemicals. Fractionation is carried out by adding biomass, the solvent mixture, and a sulfuric acid catalyst to a

3.5 L, 3" bore, pressurized, Hastelloy flow-through reactor, controlled via LabVIEW and operating at three temperatures: 120°C, 140°C, and 160°C. The solvent selectively dissolves the hemicellulose and lignin, while the cellulose fraction is obtained as a solid in an average yield of 34.9 % by weight. The recovered solvent is subjected to a phase separation, giving an organic phase containing lignin and an aqueous phase containing hemicellulose. Lignin is recovered at an average yield of 13.7 % by weight and is fairly constant and independent of temperature and pressure.

POOVAIAH, CHARLESON

Monitoring switchgrass transformation with fluorescent proteins (Charleson Poovaiah, Jason Burris, David Mann, Neal Stewart, Jr.; Department of Plant Sciences, University of Tennessee, Knoxville, TN)

Abstract: Switchgrass has the potential to be an important bioenergy crop because of its high biomass yield and cellulose content. The lignocellulosic biomass can be used for ethanol production through bioconversion. Genetic transformation can be used to modify cell wall properties, reduce its lignin content and increase cellulose content to improve the efficiency of ethanol production from the crop. Agrobacterium-mediated transformation has been successfully used to transform many monocot species. Identification and selection of transgenic callus is tedious and often takes several weeks of growing the callus under the stress of selective agents. The use of a visible selection marker would allow earlier and rapid selection of transient and stably transformed cells by fluorescence microscopy and can also be used in conjunction with antibiotic selection. A novel red fluorescent protein of DS-Red type of fluorescent protein, pporRFP from the coral *Porites porites* was used for visual screening of Agrobacterium-mediated switchgrass transformation. Transient expression of RFP could be observed three days after transformation and the growth of the transformed cells could be monitored visually.

SHPAK, ELENA D.

ERECTA mRNA splicing leads to an increase in the gene expression at the posttranscriptional level (Rebecca Wilson, Wusheng Liu, Spencer Willet, and Elena D. Shpak, Department of Biochemistry, Cellular & Molecular Biology, The University of Tennessee)

Abstract: The ERECTA receptor-like kinase and its paralogs play an important role in plant growth and development by promoting cell proliferation in the organ primordia formed by shoot apical meristem. They achieve their goal by stimulating cell cycle and in some tissues inhibiting cell differentiation. The amount of the ERECTA protein present in developing plant tissues is regulated by presence of introns in the ERECTA gene. While the presence of ERECTA introns does not affect transcription it is necessary for ERECTA protein synthesis. During splicing several proteins are deposited on mRNA 20-24 nucleotides upstream of an exon-exon junction. The exon junction complex (EJC) proteins are found exclusively on spliced mRNAs and are involved in several processes regulating mRNA metabolism. We hypothesize that splicing by recruiting the EJC to the ERECTA mRNA may be required for efficient incorporation of ERECTA into polysomes. We also predict the existence of splicing requiring sequences in ERECTA exons that distinguish ERECTA mRNA from the bulk of other RNAs that do not require splicing. Data from transient expression system where different portions of the ERECTA gene with or without introns are fused to luciferase supports our hypothesis.

Regulation of plant growth and development by ERECTA family genes in Solanaceae family and in Arabidopsis (Danyu Kong, Hector Villagarcia, Kanishka de Silva, Mariya V Khodakovskaya, and Elena Shpak, Department of Biochemistry, Cellular & Molecular Biology, The University of Tennessee)

Abstract: To understand the control of organ growth and to enable design of crops with desired size and shape of their organs we study the ERECTA family genes (hence referred to as ERLs).

ERLs regulate cell proliferation and differentiation in aboveground organ primordia. In Arabidopsis the loss of ERLs leads to production of normal primordia that later develop into dwarf organs due to reduced cell proliferation and premature cell differentiation in the epidermis. The ERLs form an ancient gene family that already existed in Bryophytes and is expected to exist in all crop species.

Most knowledge of ERL function has been obtained using Arabidopsis as a model system. Now we are trying to transfer this knowledge to Solanaceae family and specifically to tomato (*Solanum Lycopersicon*) and tobacco (*Nicotiana glauca*) and through comparison of ERL function in different plant species advance our understanding of this important signaling pathway. We expect that the function of ERLs in Solanaceae and Arabidopsis is similar with respect to regulation of cell proliferation and stomata development but might regulate shape of different organs (e.g. fruit) in a species specific manner. Using a transgenic approach we have down-regulated ERL signaling pathway in tomato and tobacco, which led to dwarfism.

SHI, ZENGQIAN AND SIQUN WANG

Preparation, Characterization and Application of Cellulose Nanofibers—a novel investigation based on high-intensity ultrasonication (Zengqian Shi and Siqun Wang, Tennessee Forest Products Center, University of Tennessee, Knoxville TN 37996-4570)

Abstract: Cellulose fibers in micro/nanoscale are potential alternatives of man-made fibers for their peerless mechanical properties, excellent biocompatibility and huge storage in natural biomass. Herein, a novel mechanical-treatment-based process was presented to isolate cellulose micro/nanofibers from different sources followed by their morphology and property characterization as well as the preliminary investigation of their applications in nanocomposites. The achievement in progress was also given here. This presentation will summarize our recent work including the following five sections:

1. A novel process was presented to isolate fibrils from several cellulose-based sources effectively by high-intensity ultrasonication (HIUS). The results implicated that high-intensity ultrasonication technique is an environmentally benign method and a simplified process that conducts fiber isolation and chemical modification simultaneously and helps significantly reduce the production cost of cellulose nanofibers and their composites;
2. Effects of process and source on elastic modulus of single cellulose fibrils were evaluated by atomic force microscopy. The results indicated that elastic modulus of Lyocell fibrils with diameters from 150 to 180 nm was evaluated to be 98 ± 6 GPa. Modulus values decreased dramatically when the diameter was more than 180 nm;
3. Poly(vinyl alcohol) nanocomposites reinforced with cellulose fibrils isolated by high intensity ultrasonication;
4. Physical and mechanical properties of polyvinyl alcohol and polypropylene composite materials reinforced with fibril aggregates isolated from regenerated cellulose fibers;
5. The ongoing investigations in preparation of cellulose nanofibers using a “green” method holding the benefits of bioproduct and biofuel.

WEI, JIANHUA

Obtainment of low-lignin content transgenic poplar by down-regulation of CCoAOMT expression (Jianhua Wei¹, Hongzhi Wang¹, Ruifen Li¹, and Yanru Song²—¹Beijing Agro-Biotechnology Research Center, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China; ²Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China)

Abstract: Lignin, along with cellulose, is a major constituent of wood with very important biological functions. During the process of paper making, lignin is chemically removed from cellulose and hemicellulose of wood in kraft pulping, which consumes large quantities of noxious chemicals and energy. In conversion of lignocellulosic biomass to bioethanol, the presence of lignin is the major factor limiting the accessibility of plant cell wall polysaccharides to chemical, enzymatic and microbial digestion, which need to be removed in the pretreatment. Genetically reducing lignin in the biomass can facilitate both of papermaking and bioconversion to biofuel. We have obtained low-lignin content transgenic poplar (*Populus tremula* × *Populus alba*) by transforming the antisense CCoAOMT into it. We assayed the expressing level of CCoAOMT and lignin content for 3 years continuously, which demonstrated the depression of CCoAOMT expression was maintained over 3 years. Then, we evaluated pulping performance of 3-year-old field-grown transgenic poplar. The transgenic poplar with anti-sense CCoAOMT had an about 13% decreased lignin content, in which a slight increment was found in S/G ratio. Chemical analysis showed that the transgenic poplar had significantly less benzene-ethanol extractive than that of control wood, but cannot detect significant differences in contents of Ash, Cold water extractive, Hot water extractive, 1% NaOH extractive, Holocellulose, Pentosans and Cellulose. Kraft pulping showed that lower lignin in transgenic poplar led to remarkable improved pulp quality and increased pulp yield. The low-lignin content transgenic poplar exhibited easier delignification in the kraft pulping. Although conditions of the kraft pulping and acid pretreatment for biofuel production are different, it could be expected that the lower lignin amount in transgenic poplar in this research is beneficial to biofuel production. The evaluation of performance of the transgenic poplar in conversion of bioconversion to biofuel is ongoing.