The 2\textsuperscript{nd} China-US Workshop on \textit{Biotechnology of Bioenergy Plants}

September 19-21, 2010
Beijing, China
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Sponsors

- Institute of Botany, the Chinese Academy of Sciences (CAS)
- The University of Tennessee
- Purdue University
- Oak Ridge National Laboratory

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- Co-Chair
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  Haichun Jing (Institute of Botany, CAS, China)
  Laigeng Li (Shanghai Institute of Physiology and Biochemistry, CAS, China)
  Yongqing Ma (Institute of Soil and Water Conservation, CAS, China)
  Richard Meilan (Purdue University, USA)
  Shihua Shen (Institute of Botany, CAS, China)
  Songquan Song (Institute of Botany, CAS, China)
  Neal Stewart (The University of Tennessee, USA)
  Jianhu Wei (Bioenergy Research Center, Beijing Academy of Agricultural Sciences, China)
  Zengfu Xu (Xishuangbana Garden, CAS, China)
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  Peng Zhang (Shanghai Institute of Physiology and Biochemistry, CAS, China)
  Jie Zhuang (The University of Tennessee, USA)
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Shihua Shen (Institute of Botany, CAS, China)
Songquan Song (Institute of Botany, CAS, China)
Zhengle Yang (Institute of Botany, CAS, China)
Jie Zhuang (The University of Tennessee, USA)
Introduction

On July 20, 2006, in Beijing, representatives of the University of Tennessee-Oak Ridge National Laboratory’s (UT-ORNL) Joint Institute for Biological Sciences (JIBS) and UT’s Institute for a Secure and Sustainable Environment (ISSE) signed a framework agreement for the establishment of a China-US Joint Research Center for Ecosystem and Environmental Change (http://isse.utk.edu/jrceec/). The focus of this agreement is to promote research collaboration, academic exchange, student education, and technology training and transfer in areas of environmental concern. This specific agreement was reached with two Institutes of the Chinese Academy of Science (CAS)—the Institute of Geographical Science and Natural Resources Research (IGSNRR) and the Research Center for Eco-Environmental Science (RCEES)—both in Beijing. The center’s primary collaborative themes include: (1) ecosystem processes and management, (2) environmental sustainability of bioenergy production, (3) ecological foundations of water resources and quality, and (4) technologies for improvement of eco-environmental systems. Since establishment of the Joint Center, a Bioenergy and Environmental Sustainability theme has arisen and been the topic of several reciprocal visits and joint workshops in Beijing, and Knoxville, Tennessee. The Joint Center convened the first China-US workshop on September 11-14, 2007, in Knoxville to address environmental aspects of bioenergy production and sustainability. About 40 scientists from the partnering institutions and six program officers from U.S. National Science Foundation (NSF) and Chinese government attended the workshop. The second China-US workshop, sponsored by the funds of U.S. National Science Foundation (NSF) and the Natural Science Foundation of China (NSFC), was held on October 15-18, 2008, in Beijing, China, with focus on bioenergy consequences for global environmental change. About 80 scientists, students, and program leaders attended the conference, including officials from U.S. Embassy in Beijing. As a follow-up activity, “China-US Workshop on Biotechnology of Bioenergy Plants” will be organized by the UT and UT-ORNL JIBS to bring together bioenergy plant researchers from China and the United States to exchange scientific findings and promote international collaboration in research and education.

Background

The China and US economies are the globally dominant drivers of fossil fuel consumption and release of greenhouse gases and are thus strategically linked to the sustainable development of alternative and renewable energy sources. China and the US are natural partners for the development of biofuels. Although they differ in some aspects of agriculture, natural resources, economy, and society, the two nations share many facets in environmental and global change issues. In the US this has given rise to a robust new rural economy of ethanol production which
is attempting to meet mandates by expanding and diversifying to non-food, cellulosic feedstocks to meet current and future demand. The US Department of Energy has completed the ‘Billion Ton Study’ indicating the need for cellulosic biomass from forest products and cultivated feedstock biomass such as Switchgrass are needed in order to achieve transportation biofuel goals over the next two decades. In China, the government and renewable energy industry are poised to capitalize on the marketing potential of biofuels. China reports that a comparable billion tons of cellulosic material may be available for biofuel production annually from agricultural wastes. China’s 21st Century Agenda emphasizes renewable energy as a foundation for development and the Medium and Long-term Development Plan for Renewable Energy targets 30 GW of biomass power based on agricultural and forestry wastes and energy crops by 2030. However biomass production is facing many grand challenges in view of limits of available natural resources (such as lands and water). Large scale production of bioenergy needs a diverse species of energy plants that are tolerant to environmental stresses and easy to breakdown in bioconversion. In this aspect, biotechnology remains huge potential for making breakthroughs in near future. Biotechnology of bioenergy plant feedstocks is currently developing very rapidly in the United States and China. For instance, drought-tolerant research on switchgrass is ongoing in northwestern China. In east China, many new species of energy plants are being studied and applied to industrial conversion for bioenergy production. It is therefore necessary to create an opportunity for key Chinese and American scientists to know each other and discuss the potential for scientific exchange, research collaboration, and joint student education.

**Workshop goals and objectives**

Through the workshop, participants will review the advances in biological research on bioenergy plants and discuss future research directions of relevant bioenergy technologies. The workshop will seek to develop joint research/education programs between China and the U.S. in the areas of plant biotechnology. Specifically, the workshop will:

- Evaluate the research progress in the biotechnology of bioenergy plant;
- Summarize the availability of energy plant species and their biomass capacity and environmental benefits;
- Explore research strategies for breakthrough of key bioenergy biotechnology;
- Develop a student/faculty professional network that facilitates bioenergy science exchanges, site visits and investigations, and industry/government engagement in China and the US.
Welcome to 2nd China-US Workshop

We are very glad that the 2nd topical US-China Workshop on the Biotechnology of Bioenergy Plants will be taking place from Sunday, September 19 to Tuesday, September 21, 2010 in Beijing, China.

As a follow-up activity of the first topical workshop that was held November 2009 in Downtown Knoxville, Tennessee of the United States, the theme for this year’s workshop will continue to be biotechnology with focus on reviewing the advances in scientific research on bioenergy plants, discussing future research directions of relevant bioenergy technologies, and seeking to develop joint programs of research and education between China and the U.S. in the areas of plant biotechnology.

Beijing is the world-famous ancient cultural city. It has the long history of over 3,000 years, with more than 850 years for the imperial capital. Beijing has rich natural and tourism resources—the impressive world geological park, fantastic museums, esteemed ancient mausoleums, magnificent imperial palace, fabulous imperial parks, sacred temples, vibrating ancient towns and villages, graceful towers, modern commercial streets and the vast national forest parks, etc. Among all the popular attractions, the most famous attractions in Beijing include the Great Wall, Forbidden City, Tiananmen Square, Temple of Heaven, Summer Palace, Prince Gong’s Mansion, Lama Temple, Ming Dynasty Tombs and Olympic Sites (Bird’s Nest and Water Cube). It attracts tens of millions of visitors and tourists both at home and abroad each year to enjoy its rich culture and wonderful scenery.

We are look forward to seeing you at this topical workshop in Beijing. Your participation is highly appreciated. We sincerely hope that you will enjoy the meeting and your stay in Beijing.
Map of Workshop and Accommodation Location

Workshop location
Multi-function Hall, Library Building, Institute of Botany, the Chinese Academy of Sciences (中国科学院植物研究所图资楼二楼多功能厅)

Accommodation location
Fragrant Hill Empark Hotel (北京香山金源商旅中心酒店)
Add: No. 59 North Zheng Huang Qi, Haidian District, Beijing 100093, China
Tel: 86-10-59898888
Fax: 86-10-62595959
Website: http://www.empark.com.cn/english/bj03/jdjs.asp?id=645

Accommodation location
Beijing Siji Royal Garden International Hotel (北京四季御园国际大酒店)
Add: No. 168 Hanhe Rd, Haidian District, Beijing 100093, China
Tel: 86-10-59325566, 88857777
Website: http://blog.sina.com.cn/wujuanhotels
Agenda

Saturday, September 18, 2010

09:00 - 18:00  Registration and Check in
Room 217, Library Building, Institute of Botany, the Chinese Academy of Sciences (CAS)

18:00 – 20:30  Dinner

Sunday, September 19, 2010

09:00 – 09:40  Welcome and Introduction
Moderator: Gongshe Liu

09:00  Welcome and opening remarks by Tingyun Kuang (Institute of Botany, CAS)
09:10  Introduction about China-US Joint Research Center by Gary Sayler (The University of Tennessee (UT), USA)
09:20  Welcome addresses by the representatives of Ministry of Science and Technology, National Natural Science Foundation of China and CAS

09:40 – 10:00  Workshop Group Picture & Coffee Break

09:40 – 11:55  Keynote Addresses on Multidisciplinary Approach to Bioenergy (I)
Moderators: Shihua Shen

10:00  “Bridging Plant Biotechnology for Broad Applications of BioEnergy Biomass in Environmental Sustainability” by Gary Sayler (UT, USA)

10:40 – 11:55  Plenary Addresses on Novel Biotechnology for Biofuels
Moderators: Jeffrey Volenec and Laigeng Li

10:40  “Applied Photosynthesis-Putting Photosystem I to Work” by Barry Bruce (UT, USA)
11:05  “Advanced Biofuels Production through Metabolic Engineering” by Feng Chen (UT, USA)
11:30 “Grass Genomic Resources and Application in Bioenergy Plant Improvement” by Gongshe Liu (Institute of Botany, CAS, China)

12:00 – 13:30 Lunch and Poster Presentations

13:30 – 15:10 Plenary Addresses on Production of Biomass (I)

Moderators: Fang Chen and Sylvie Brouder

13:30 “Development Mode of Large-scale production of Jatropha curcas as Biodiesel Feedstock” by Fang Chen (School of Life Sciences, Sichuan University, China)

13:55 “Water Use Efficiency in Bioenergy Crop Production” by Jeffrey Volenec (Purdue University, USA)

14:20 “Induction of Orobanche Seeds Germination by Switchgrass (Panicum virgatum L.) at Different Grow Periods” by Yongqing Ma (Institute of Soil and Water Conservation, CAS, China)

14:45 “Biomass Production of Switchgrass (Panicum Virgatum L.) in the Semiarid Loess Plateau of China” by Bingcheng Xu (Institute of Soil and Water Conservation, CAS, China)

15:10 – 15:30 Coffee Break

15:30 – 17:10 Plenary Addresses on Genetic Engineering of Herbicious Bioenergy Crops

Moderators: Guojiang Wu and Barry Bruce

15:30 “Switchgrass Biotechnology and Modifications for Improved Bioenergy Feedstocks” by David Mann (UT, USA)

15:55 “Sequence Polymorphism and Structural Variation in Genomes of Sorghum bicolor” by Haichun Jing (Institute of Botany, CAS, China)

16:20 “Biotechnological Starch Modification in Cassava and Sweet Potato for Industrial Applications” by Peng Zhang (Institute of Plant Physiology and Ecology, CAS, China)

16:45 “Advances in Switchgrass Resistance Study and Its Application to the Biomass Production from Marginal Lands” by Haitao Zuo (Beijing Academy of Agriculture and Forestry Sciences, China)

17:10 – 20:00 Reception

Monday, September 20, 2010

09:00 – 10:20 Keynote Addresses on Multidisciplinary Approach to Bioenergy (II)
Moderator: Feng Chen

09:00 “Rapid Determination of Woody Biomass Digestibility and Chemical Composition Using Near-Infrared Spectroscopy” by Laigeng Li (Institute of Plant Physiology and Ecology, CAS, China)

09:40 “Center for Renewable Carbon: Advancing the Green Economy” by Tim Rials (UT, USA)

10:20 – 10:35 Coffee Break

10:35 – 11:50 Plenary Addresses on Production of Biomass (II)
Moderators: Tim Rials and Haichun Jing

10:35 “Nitrogen Use Efficiency in Bioenergy Cropping Systems” by Sylvie Brouder (Purdue University, USA)

11:00 “Characterization of Miscanthus Germplasm Collection in China” by Zili Yi (College of Bioscience & Biotechnology, Hunan Agricultural University, China)

11:25 “Establishing a Feedstock Supply Chain for Cellulosic Ethanol in Tennessee” by Sam Jackson (UT, USA)

12:00 – 13:30 Lunch and Poster Presentations

13:30 – 14:20 Plenary Addresses on Genetic Engineering of Woody Bioenergy Plants
Moderators: Zengfu Xu and Sam Jackson

13:30 “Evolution and Divergence in the Coding and Promoter Regions of the Populus Gene Family Encoding Xyloglucan Endotransglycosylase / Hydrolases” by Max Cheng (UT, USA)

13:55 “Genetic Modification of Poplar with Low Lignin Content for Ethanol Production” by Jianhua Wei (Beijing Academy of Agriculture and Forestry Sciences, China)

14:20 “Proteomic Analyses of the Seed Development of Jatropha curcas” by Hui Liu (Institute of Botany, CAS, China)

14:20 – 14:40 Coffee Break

14:40 – 15:55 Plenary Addresses on Genetic Engineering of Woody Bioenergy Plants
Moderators: Max Cheng and Jianghua Wei

14:40 “Cloning and Functional Characterization of Fatty Acid Biosynthesis-related Genes from Jatropha curcas” by Guojiang Wu (South
China Botanical Garden, CAS, China)

15:05 “Increasing Seed Yield in Biofuel Plant *Jatropha curcas* by Manipulating Floral Development and Sex Expression” by Zengfu Xu (Xishuangbanna Tropical Botanical Garden, CAS, China)

15:30 “*Jatropha* and Sweet Sorghum as Efficient Bioenergy Plants” by Srinivasan Ramachandran (Temasek Life Sciences Laboratory and China-Singapore Joint Lab for Energy Plant)

15:55 – 17:30 **Group Discussion on International Collaboration and Student/Faculty Exchange**

Moderator: Gongshe Liu and Feng Chen

Topics include the publication of 2nd workshop results and the plan for next year’s workshop.

17:30 - 17:45 **Concluding Remarks and Workshop Ends**

by Tingyun Kuang (Institute of Botany, CAS, China)

18:00 - 20:30 **Dinner**

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**Tuesday, September 21, 2010**

09:00 - 10:30 **Visit to Institute of Botany, CAS, China**

Coordinator: Songquan Song

10:30 - **Local tour in Beijing**
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Cloning and functional characterization of fatty acid biosynthesis-related genes from jatropha curcas ..............................................................................................................33

Increasing seed yield in biofuel plant Jatropha curcas by manipulating floral development and
sex expression .........................................................................................................................34

Jatropha and sweet sorghum as an efficient bio-energy plants .............................................35
Bridging plant biotechnology for broad applications of bioenergy biomass in environmental sustainability

Gary S. Sayler *

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During the historical course of traditional agriculture, crop production focused on successfully increasing crop yields and nutritional quality with investment in intensive labor, mechanization, irrigation, fertilization, pest and disease control. This success in meeting humankind’s basic needs did not come without environmental costs and future energy biomass crop deployment needs to reverse these costs where possible. Future demands can provide opportunities for many different kinds of biotechnical improvements to support both energy biomass development and lessening the impact of environmental change.

Plant modifications through conventional selection or genetic engineering are needed to develop future energy biomass sources that can be economically feasible for large scale production of transportation fuels and byproducts while simultaneously buffering environmental change and promoting sustainability. The goal of improving plant biomass for ease of cellulose conversion to fermentable carbohydrates substrates and lignin conversion to phenolic feedstocks, or enhanced production of usable oils needs to be complemented with forward thinking applications for environmental restoration and needs for global scale life cycle analysis and greenhouse gas reduction.

In the USA more than 40 million acres of pasture and marginal cropland are needed for production of energy biomass and an equivalent amount of forest may be required to achieve energy and transportation fuel security goals of the near future. Many million more acres will be needed on a global scale as individual societies struggle to replace fossil carbon energy sources while maintaining food security. The energy crops of the future may well require improved drought, salt, metal, and thermal stress tolerance, reduced fertilizer input and greater pest resistance to broaden their growing range on marginal lands with reduced production inputs. However, sustainability may drive more dramatic changes needed in photosynthesis and carbon allocation pathways for root exudation and plant modulation of the microbial rhizosphere biomass and activity. Such ecological control may be required to achieve carbon offsets through mid term storage in below ground biomass and stable soil carbon.
**Biosketch:** 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.

**Applied photosynthesis- putting photosystem I to work**

Barry Bruce*

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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 separate biomolecular structures that have become specialized...
to either capture photons and facilitate rapid energy transfer via exciton coupling of pigments or in a separate structure, known as a reaction center, to convert this photon/exciton into a charge separation. Both of these processes function with a quantum yield approaching unity. Through natural diversity the light harvesting process has adapted to capture a wide range of the visible solar energy. Although both the pigments and organization of these light-harvesting complexes demonstrate considerable diversity, the charge separation process is fundamentally conserved in reaction centers. Drawing on the remarkable efficiency, stability, and renewability of these biological complexes, we have begun to directly exploit their properties to 1) act as luminescent solar concentrators, 2) function as biophotovoltaic devices and 3) serve as a light driven catalyst for hydrogen production. I will report on the design and fabrication of these bio-hybrid devices. In addition, I will discuss future designs to further enhance their EQE towards the goal of a truly sustainable and environmentally benign new strategy for bioenergy production. This work has been supported by grants from the NSF Nanoscience Interdisciplinary Research Team Program, NSF IGERT, NSF Program in Sustainable Science, the Gibson Family Foundation, and UTK SEERC.

Biosketch: 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
Advanced biofuels production through metabolic engineering

Feng Chen*, Nan Zhao

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Advanced biofuels are, in essence, biochemically with fuel properties. Understanding the biochemical pathways leading to various potential biofuels and the improvement of their production through metabolic engineering are the drive force of advanced biofuels. This talk will discuss two distinct biochemical pathways, the terpenoid pathway and the fatty acids pathway, both leading to the production of biodiesels. Terpenoids constitute a very diverse class of secondary metabolites. Many organisms, especially plants, make terpenoids in large quantities. Terpenoid biosynthesis offers a rich source of advanced biofuels. With the ability to produce branched-chain and cyclic alkanes, alkenes and alcohols of different sizes with diverse structural and chemical properties, the terpenoid pathway could produce fuels or precursors to gasoline and biodiesel. In this talk, the chemical properties of terpenoids and the potential of using terpenoids as a form of renewable biodiesel will be first introduced. Our current understanding of terpenoid biosynthesis and genomic approaches that can be used for elucidating the molecular machinery of terpenoid biosynthesis will be presented. Novel strategies of using metabolic engineering to create novel bioenergy crops based on the terpenoid pathway will be discussed. For the second biochemical pathway that will be presented, a novel discovery of the Chen lab that can be used for direct production of biodiesel, i.e. fatty acid esters, through metabolic engineering, will be introduced and discussed. In short, the key message that will be delivered through this talk is that metabolic engineering is a driven force of advanced biofuels.

Biosketch: 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 as an Assistant Professor at the University of Tennessee in September 2004 and was promoted to Associate Professor with tenure in July 2010. 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.

**Comparative analysis of genomic resource in seven representative gramineous grasses**

Xiaoxia Li, Xuejun Yan, Shuangyan Chen, Xingyong Ma, Xianjun Peng, Xiaofeng Li, Gongshe Liu*

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With the completion of genome sequencing in a number of Gramineae family species, comparative analyses can be used to produce and publish data sets that facilitate identification of conserved and characteristic resources among species. Expressed sequence tag (EST) data are a major contributor to the known plant sequence space. In this paper, we review status of genomic resources of seven representative gramineous grasses, *Brachypodium distachyon*, *Panicum virgatum* (switchgrass), *Festuca arundinacea* (tall fescue), *Cynodon dactylon* (bermudagrass), *Agrostis stolonifera* (bentgrasses), *Lolium perenne* (perennial ryegrass) and *Leymus chinensis* (Yang grass). Furthermore, comparative analysis of genomic resource among a model system *Brachypodium distachyon* and other six representative gramineous grasses, will allow us to grasp the phylogenetic relationship and the biological properties of each species and to accelerate discovery of stress resistance genes, the genes related to different organs and the specific genes in different developmental stages, which can be used for improvement of biomass crop against the stress; especially find the genomic resources to resist to future climate changes.

**Biosketch:** Prof. Gongshe Liu got his Ph.D. degree from Blaise-Pasgal University and CNRS of France in 1986. After that, he started his post-doctoral research, and then has been working in Institute of Botany, Chinese Academy of Sciences. Now he is PI for GENE RESOURCES of strategic Plants in IB-CAS.

Prof. Liu has been studying *L. chinensis* over fifteen years. His group collected more than thousands accessions of *L. chinensis* from China, Mongolia and Korea. These materials had a wide range of genetic variation. He found that *L. chinensis* was a self-incompatible species. 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 identify new genes from *L. chinensis* related with high biomass and adverse resistance as well as genes regulation, which will provide the fine gene resources for the molecular design breeding of forage
Development models of scale production of jatropha biodiesel feedstocks

Fang Chen *, Ying Xu, Lin Tang, Jun Wu

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Jatropha curcas is resistant to drought, high temperature, barren soil and insects, and thus, can be cultivated on barren hills and wasteland, not competing lands with food, not competing food with people. The seed of Jatropha curcas contains a great amount of oil, generally from 25% to 35%, and is rich in oleic acid and linoleic acid (over 70%), and is especially suitable for feedstocks of biodiesel. In addition, seeds, leaves and other parts of this plant have many valuable chemical elements, and the tree is a versatile economic tree. Myanmar and other countries, plan to replace poppy cultivation Park by planting jatropha trees. Thus, the potential for widespread use of J. curcas as a biodiesel feedstock is widely recognized.

Jatropha curcas is mainly distributed in tropical, subtropical and dry and hot valley. In china, it is mainly distributed in Sichuan, Yunnan, Guizhou, Hainan, Guangdong, Guangxi etc. provinces. At present, domestic and foreign have a certain scale jatropha cultivation of raw materials. China has approximately 0.2 million hectares of jatropha forest, and more than 3 million hectares of fitting wastelands. The present researches show that the scale production of jatropha biodiesel materials are looking forward to mainly solve following issues: the need for developing more fine varieties, large-scale planting patterns of raw materials, large-scale harvesting and processing technology, large-scale production compared costs and benefits analysis, etc.. In some countries, the large-scale production mode of bio-energy raw materials is relatively mature, such as the use of rape in Germany, soybeans in United States and sugar cane in Brazil, palms in Malaysia. But, these models are not applicable to the scale production of jatropha biodiesel raw materials in China. Currently, large-scale jatropha feedstock production patterns are: Indonesia and other Southeast Asian countries have adopted “land + company + farmers” model; Mali and other African countries have adopted “international organizations + farmers” model; Brazil and some South American countries have adopted “the homesteader + company” model; China is mainly
Water-use efficiency of biomass cropping systems

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In arguments on the relative merits of potential biofuel crops, water is a critical, but often over-looked determinant of system viability. Current economic analyses predict that the cost to purchase and operate supplemental irrigation for biofuels cropping systems will significantly reduce NEB and economic returns making irrigation an unlikely management option for most biomass production systems. Biomass yield is closely related to water uptake, a relationship often described as WUE. Values for WUE range from 400 to >1500 kg water/kg dry biomass. The WUE varies with plant photosynthetic mechanism, with C₄ plants having higher WUE than C₃ plants. Even within C₄ species important differences in WUE also exist, with sorghum often having higher WUE than maize. The superior drought and heat tolerance of sorghum, and its stable WUE, highlights its potential advantage among annual C₄ species for biofuels production. For perennials meta-analysis has indicated Miscanthus yield to be more strongly influenced by precipitation than yield of switchgrass. Although Miscanthus is higher yielding in high rainfall environments, switchgrass may be better adapted to many of the water-limited regions not suited for grain crop production that are being considered for herbaceous biofuels production. Recent modeling efforts suggest that Miscanthus productivity across Europe will be constrained by water availability. Miscanthus species may differ in WUE, with M. sacchariflorus have greater WUE than M. giganteus; however, there studies were with potted plants in controlled conditions and results should be interpreted with caution. Breeding for high WUE has been attempted in several species used for forage/biomass and most have reached similar conclusions; selection for high yield alone was as effective as selecting for WUE per se. In addition, factors that reduce yield like low fertility/nitrogen, similarly reduce WUE. However, few studies have explored WUE of candidate
biomass species using currently proposed management systems. Thus, there is a clear and urgent need to develop a comprehensive understanding of the water budgets necessary for biomass production before pursuing the large-scale biomass agenda that has been proposed for the U.S.

**Biosketch:** Dr. Jeff Volenec is a professor in the Department of Agronomy at Purdue University. Volenec received his M.Sc. and Ph.D. degrees at the University of Missouri-Columbia in 1983 specializing in crop physiology where he studied leaf growth and development in grasses. He earned his B.Sc. in Agronomy/Natural Sciences at the University of Wisconsin-Madison. His appointment at Purdue encompasses all three areas of the Land Grant mission including teaching, research, and Extension. In addition, he served as Associate Head in the Agronomy Department from 1993 to 2009. Volenec’s research focuses on the biochemistry, physiology and ecology of perennial plants used for forage and biomass. His teaching responsibilities have included upper-division courses in Crop Physiology and Ecology, and Forage Management. He is a five-time recipient of the Outstanding Teaching Award in the Department of Agronomy at Purdue University. Students also have twice selected him as Outstanding Counselor in the Department of Agronomy. He recently completed a six-year term as Chair of the Graduate Program in the Department of Agronomy, including leadership roles in administration of Graduate Education Programs in the College of Agriculture and in the Graduate Council of Purdue University. He has served as Editor of *Crop Science* and as Editor-in-Chief of the Crop Science Society of America. He is the recipient of Purdue University’s Agricultural Research Award and the Young Crop Scientist Award from the Crop Science Society of America. He currently serves as a Non-Resident Fellow for the Noble Foundation. For his accomplishments, he has been elected Fellow of the American Association for the Advancement of Science, the American Society of Agronomy, and the Crop Science Society of America. He is currently Incoming President – elect of the Crop Science Society of America.

**Induction of Orobanche seeds germination by switchgrass (Panicum virgatum L.) at different grow periods**

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Orobanche spp. is parasitic weeds in agriculture and it is difficult to control because of its special life cycle. In preliminary study we found that the water and methanol extracts of switchgrass root and shoot are able to induce *Orobanche cumana* seeds to germinate. *O. cumana* is a problem in sunflower field and causes severe yield loss in Xinjiang, Jilin, Hebei and Shanxi provinces. Generally speaking, sunflower is planted in the harsh area, where the environment is dry and the less fertile soil. In such area, switchgrass normally grow well. In this study, rhizosphere soil, root and aerial parts extracts of nine cultivars of switchgrass (Alomo, Blackwell, Cave-in-Rock, Forestberg, Illinois USA, Kanlow, Nebraska 28, Pathfinder, Sunburst) were subjected to stimulating germination of *Orobanche* (*O. minor*, *O. cumana*, *O. aegyptiaca*) seeds. The cultivars, which induce higher germination rate of *Orobanche* seeds, may be selected as "trap crops" to control Orobanche. Results indicated that switchgrass was not only able to stimulate
germination of *O. minor* seeds, but also has the potential to induce germination of *O. cumana* and *O. aegyptiaca* seeds. Rhizosphere soil of 21 days old Nebraska 28 stimulated germination of *O. cumana* and *O. aegyptiaca* seeds at the rate of 23.3% and 25.3%, respectively. The water extracts of switchgrass aerial parts induced *O. cumana* seed germination at a higher rate than *O. aegyptiaca*. The maximum germination rate (58.7%) of *O. cumana* occurred for the cultivar of Forestberg, Nebraska 28 could induce a germination rate up to 50.0% for the same *Orobanche* seeds. Switchgrass in rhizosphere soil for 42 days growth could induce germination of *O. cumana* seed. In the initial stage (growth of 21 days), the roots and aerial parts extract of switchgrass tend to induce seed germination of *O. aegyptiaca*. In addition, the methanol extract of aerial part of Alomo after growth of 61 days were able to stimulate *O. aegyptiaca* germination at a rate of up to 54.0%, and the root extract also could induce seed germination with a rate of 40.3%.

**Biosketch:** 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. 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, *Stellera 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 bermouthica* (Del.) Benth germination stimulant(s) from *Menispermum dauricum* (DC.) root culture and effect of medium composition on production of *Striga bermouthica* (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.

**Biomass allocation, relative competitive ability and water use efficiency of two native species in semiarid Loess Plateau under water stress**

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A better understanding of the growth and interspecific competition of native species under water stress will facilitate the prediction of the community succession and to choose appropriate approaches for conservation and artificial use in drought environment. Biomass production and allocation, relative competitive ability and water use efficiency of one C₄ herbaceous grass, *Bothriochloa ischaemum* and one C₃ leguminous subshrub, *Lespedeza davurica* from the semiarid Loess Plateau of China were investigated in a pot-cultivated experiment under different levels of water availability. The experiment was conducted using replacement series design in which *B. ischaemum* and *L. davurica* were grown with twelve plants per pot, in seven combinations of the two species (12:0, 10:2, 8:4, 6:6, 4:8, 2:10, and 0:12). Three levels of water treatments included sufficient water supply (HW), moderate water stress (MW) and severe water stress (LW) were applied after seedling establishment till the end of the experiment. Biomass production and its partitioning, and transpiration water use efficiency (TWUE) were determined at the end of the experiment. Interspecific competitive indices (competitive ratio (CR), aggressivity (A) and relative yield total (RYT)) were calculated from the biomass dry weight data for shoots, roots and total biomass. Water stress decreased biomass production of both species in monoculture and mixture. The growth of *L. davurica* was restrained in their mixtures for each water treatment. *L. davurica* had significantly (P<0.05) greater root/shoot ratios than *B. ischaemum* for each water treatment and proportion within the replacement series. Aggressivity (A) values for *B. ischaemum* with respect to *L. davurica* were negative only at the proportions of *B. ischaemum* to *L. davurica* being 8:4 and 10:2 in LW treatment. *B. ischaemum* had a significantly (P<0.05) higher CR value under each water treatment, and water stress considerably reduced its relative CR while increased that of *L. davurica*. RYT values of the two species indicated some degree of resource complimentarity under both water sufficient and deficit conditions. The results suggest that it is advantageous for growing the two species together to maximize biomass production, and the suggested ratio was 10:2 of *B. ischaemum* to *L. davurica* because of significantly higher (P<0.05) RYT and TWUE under low water availability condition.

**Biosketch:** Dr. Xu is an associate professor working in State Key Laboratory of Soil Erosion and Dryland Farming on 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 in Graduate School of Chinese Academy of Sciences in Beijing and Institute of Soil and Water Conservation, CAS and MWR, and holds his MS in Ecology in 1999. After graduation, he jointed Institute of Soil and Water Conservation, and in 2003, he received his Ph.D. in Soil Science from Northwest A & F University.

Dr. Xu's research focuses on the physio-ecological adaptation of plants to the semiarid environment on Loess Plateau, issues including primary production, water consumption, drought resistant traits, and most recently, species interaction. Since 2000, he took switchgrass as one of the main investigated species, to study its seed germination,
seedling growth and biomass allocation under different water availability and mixed with other species such as alfalfa, milkvetch and sainfoin under different site conditions. The aims are to evaluate switchgrass eco-adaptation to the environment and obtain related field measures to use and extend it properly.

Switchgrass biotechnology and modifications for improved bioenergy feedstocks

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Switchgrass (Panicum virgatum L.) is a leading candidate feedstock for biofuels in the U.S. and is a crucial feedstock component of the BioEnergy Science Center (BESC). Improvements in biotechnology of switchgrass are important for screening potential cell wall biosynthesis genes, and genetic transformation of switchgrass is being performed by five laboratories in three institutions within BESC. Within our lab, goals and milestones have included improving tissue culture and transformation systems, isolating novel switchgrass promoters, developing a new versatile vector set for monocot transformation, and altering lignin and cellulose biosynthesis within switchgrass. Additionally, switchgrass cell suspension cultures have been produced and characterized for mutant selection, mass propagation, and gene transfer experiments via protoplast isolation for cell wall trait assessment. In order to coordinate gene expression within the BESC switchgrass transformation labs and to facilitate more rapid screening of genes, we have developed a Gateway-compatible transformation vector set (termed “pANIC”) for overexpression and RNAi-mediated knockdown for use in switchgrass and other monocot species. Overall, BESC has facilitated the coordination of scientific expertise and research in switchgrass biotechnology that would have been otherwise impossible by one investigator with funding under a traditional grant.

Biosketch: Dr. David Mann received his Ph.D. in Microbiology from the University of Tennessee in 2008 under the supervision of Gary Sayler. His work specialized in the development of novel methods for monitoring real-time single cell expression using RNAi-mediated silencing and nanotechnological approaches. His post-doctoral training occurred at the University of Tennessee Institute of Agriculture (UTIA) where he was involved in the advancement of biotechnological tools for switchgrass transformation. Currently, Dr. Mann is continuing this work as a research scientist in Neal Stewart's laboratory and has managed the bioenergy group within the lab as a part of the U.S. Department of Energy funded BioEnergy Science Center, which mainly focuses on developing improved systems for switchgrass tissue culture and transformation. The ultimate goal of this research is to produce next generation bioenergy feedstocks with enhanced properties including higher biomass yields and reduced recalcitrance for the biorefinery process.
Sequential and structural variations in the genomes of sweet sorghum

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Chinese government is committed to tackle the energy crisis by strengthening research on biofuel crops. This is well reflected in the recently released ‘China White Book of Energy Status and Strategies’ and ‘the National Energy Laws’. In the mid- and long-term energy roadmap, renewable energy will increase to occupy 15% of the total national energy supply, equivalent to 10 million ton crude oil by 2020.

Sweet sorghum (*Sorghum bicolor* Moench) is a C₄ plant with a high photosynthetic rate, high biomass yields potential, tolerance to water and saline stresses as well as low water and nutrient requirements. It can widely grow in different ecological and climate conditions. More significantly, it contains high amounts of directly fermentable stalk sugars and combustible fibres for biofuel production. Thus, sweet sorghum is high on the priority list of energy crops in China.

Sorghum is a global crop and has a long history of cultivation. Vast genetic resources exist worldwide. In the past, many useful sorghum varieties have been generated through conventional breeding programmes. The genome size of sorghum is estimated as 770Mb arranged in 2n=20 chromosomes. Many useful molecular genetic and genomic tools have been developed including an integrated genetic, physical and cytogentic map of the sorghum genome, BAC libraries, transgene technique such as agrobacterium-mediated transformation of immature embryos. Recently, large-scale shotgun sequencing of sorghum genome has been initiated through the DOE-JGI Community Sequencing Program. All these tools will greatly facilitate the molecular genetic design of sweet sorghum for bioenergy production.

We carried out resequencing of three sorghum genomes and compared the obtained information with the reference genome. Large sequential and structural variations were obtained. During my presentation, I will introduce our recent analysis on the results and discuss how the data can help improve sweet sorghum genetically to achieve the ultimate goal of developing sweet sorghum into a sustainable energy crop.

Biosketch: Dr Haicun Jing obtained his Ph.D at University of Groningen, worked in PRI and University of...
Biotechnological starch modification in cassava and sweet potato for industrial applications

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Cassava and sweet potato starches have wide-range of bioindustrial applications because of their unique property such as low levels of fat, protein, phosphorus etc. Both are also the cheapest starch sources for bioethanol production. To further extend their application potential, development of novel types of starches with different amylose/amylopectin ratios is demanding. In plants, amylose is synthesized by granule bound starch synthase I (GBSSI) and amylopectin is by starch branching enzyme SBEs. Down-regulation of GBSSI or SBE expression in cassava and sweet potato could result in reduced or increased amylose content. More than 50 transgenic cassava plant lines and hundreds of transgenic sweet potato lines were produced to express hair-pin dsRNAs homologous to their GBSSI conserved region, respectively, under the control of CaMV 35S or vascular-specific promoter p54/1.0. The plant lines were verified by Southern blot analysis for transgene integration and RT-PCR for GBSSI expression using in vitro plants.

Iodine-staining of storage roots and starch granules of several selected transgenic plant lines harvested from field demonstrated a significant reduction of amylose content both in transgenic cassava and sweet potato. Less than 1% of amylose was detected in several cassava and sweet potato lines. Analyses of GBSSI protein level by SDS-PAGE and enzymatic activity by zymogram analysis showed the decrease of GBSSI expression among these transgenic lines. Although their starch morphology did not detected significant changes by scanning electron microscopy in comparison with the wild-type’s, the inner crystal structure of starch granules of transgenic lines was different from the untransformed by transmission electron microscopy, which is further illustrated by the morphology of iodine-starch complex. The alternation of starch property from transgenic lines was also proved using differential scanning calorimeter and X-ray diffraction data. In parallel, transgenic cassava and sweet potato with down-regulated
expression of SBEI and SBEII showed increased amylose content in many transgenic lines. Our study provides a biotechnological tool to improve the starch quality in cassava and sweet potato, hence promoting their industrial utilization.

**Biosketch:** Dr. Zhang is a professor of Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Executive Director of SIBS-ETH Shanghai Center for Cassava Biotechnology and PI of Chenshan Plant Science Research Center. Prof. Peng Zhang received his Ph.D. degree in plant biotechnology from the Swiss Federal Institute of Technology Zurich (ETH-Zurich), Zurich, Switzerland, under the supervision of Prof. Nikolaus Armhein and Ingo Potrykus in 2001. After his PhD study, he joined Prof. Wilhelms Gruissem Group at ETH Zurich as a senior Researcher leading the cassava research group. In July 2005 he was recruited into the “One Hundred Talent Program” of Chinese Academy of Sciences (CAS) and became a full professor with Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, CAS. His research interests are in the fields of cassava and sweetpotato biotechnology.

**Advances in switchgrass resistance study and its application to the biomass production from marginal lands**

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One of China's strategies to reduce polluting emissions from the electricity, transport and industrial sectors is to utilize the cellulosic biomass produced from marginal lands as a sustainable source of energy. In order to help gain the necessary information for sustainably growing switchgrass as an feedstock source, a series of studies were conducted in Beijing areas aimed at assessing the potential of switchgrass as bioenergy producers and the related problems in Northern China where the marginal lands are usually characteristic of drought, salinity, leanness and poor soil structure. The resistance abilities of switchgrass to the above environmental adversities were evaluated and some demonstration plantation plots in suburban marginal lands were presented. All the aspects related to cultural practices, energy input, labor, cash and non-cash costs were also analyzed.

**Biosketch:** Dr. Haitao Zuo was previously an associate professor of Beijing Academy of Agriculture and Forestry Sciences and is currently the Executive Director and Chief Technology Officer of Beijing Friend New Energy Co. Ltd. He obtained his Ph.D. from Auburn University, USA in 2001 and then had 2-year postdoctoral research at Texas A&M University, USA. His research mainly focuses on cellulosic feedstock production from marginal lands of north
China, including variety evaluation, cultivation technologies, and biomass quality management.

**System establishment for high throughout lignocellulosic biomass analysis**

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High through-put determination of the properties of lignocellulosic material is highly desirable for biomass production and utilization. Woody biomass materials were recorded in NIR spectrum as well as determined for their chemical compositions of Klason lignin, \(\alpha\)-cellulose, holocellulose, lignin syringyl/guaiacyl (S/G) ratio and enzymatic digestibility. Fitting of the NIR information with chemical properties and digestibility by partial least-squares (PLS) regression generated a group of trained NIR models that were able to be used for rapid biomass measurement. Applying the models for woody biomass measurements led to a reliable evaluation of the chemical composition and digestibility, suggesting the feasibility of using NIR spectroscopy in the rapid characterization of biomass properties.

**Biosketch:** Prof. 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 an assistant professor and at North Carolina State University as an associate professor. Prof. Li has been studying cell wall biosynthesis for more than 16 years. His research interest focuses on the molecular biology and genomics of cell wall formation. His research has provided important insights to elucidation of the biosynthesis of cell wall polymers and opened a window to modification of cell wall for more efficient utilization of lignocellulosic biomass.

**The Center for Renewable Carbon: Advancing the green economy**

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Widespread recognition of the need for reduced dependence on petroleum has necessarily focused attention on alternatives to fossil carbon, including the renewable carbon afforded by
lignocellulosic biomass. Already the foundation of expansive economic activity, the forest resource provides valuable products from building materials to paper and plastics to chemicals, and plays a vital role in carbon sequestration. Today, new production systems are being developed to provide chemically similar lignocellulosic raw material through agricultural approaches, as well as innovative forest management practices. The likely increases in supply of biomass, and the subsequent availability of carbohydrates and lignin, make it possible to consider utility beyond liquid fuels. The University of Tennessee’s Institute of Agriculture has built an extensive program of research and development to advance technologies that provide novel fuels, chemicals, and materials from lignocellulosic biomass. Coordinated through the recently established Center for Renewable Carbon, the program engages faculty from multiple departments to coalesce the skills and backgrounds needed to address the complex challenges presented by this new direction for the institute. The presentation will discuss the mission and goals for the CRC, and will highlight several key research programs that have been advanced by virtue of its unique structure.

**Biosketch:** A native of McComb, Mississippi, Tim Rials received his undergraduate degree in Forestry from Mississippi State University in 1980. He then transferred to Virginia Tech where he earned both Masters (1983) and Ph.D. (1986) degrees from the Department of Wood Science and Technology. Tim joined the faculty at the University of California-Berkeley as assistant professor, conducting research on renewable materials. In 1988, he moved to Louisiana accepting a research scientist position with the USDA-Forest Service, Southern Research Station in Pineville, and later serving as Project Leader of the wood utilization research unit. Five years later he moved to The University of Tennessee, accepting the position of Professor in the Department of Forestry, Wildlife, and Fisheries, and Director of the Forest Products Center. At UT, Tim expanded his research into vibrational spectroscopy of wood and related materials while working to coordinate the Forest Products Center's overall research program and vision. In 2005, he assumed the role of director for the Southeast Sun Grant Center, effectively broadening the research effort to consider bioenergy, biofuels, and bioproducts. Dr. Rials was named director of Bioenergy Research in Spring 2007, and assigned to the newly created Office of Bioenergy Programs in the Institute of Agriculture. A fellow in the International Academy of Wood Science and member of the American Chemical Society, Tim continues working today to advance the efficient use of wood and lignocellulosic biomass.

**Nitrogen use efficiency in bioenergy cropping systems**

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A key factor in determining the net energy balance (NEB) and environmental footprint of any
potential biomass production scheme is the N economy of the system. Recent reviews have identified high nitrogen use efficiency (NUE) and efficient nutrient recycling as key traits needed to sustain biomass yield and also suggest that NUE of perennials may be greater than that of annual biomass species, in part, because of reduced N losses. While biomass yield of grasses is clearly driven by N fertilization, use of exogenous N significantly reduces system NEB because of the high energy input into fertilizer N production. Furthermore, few other aspects of herbaceous biomass production are more unsettled and fragmented than are the current reports and recommendations pertaining to N fertilization of perennial grasses; published results suggest response to N application varies with species, and in time and space, and literature reports of theoretically high NUE in novel bioenergy crops such as Miscanthus also show significant (e.g. 40%) fertilizer N losses from the plant-soil system. Without closer analysis of N budgets and soil N losses, it appears premature to conclude any inherent advantages in NUE for Miscanthus and other less-studied cropping systems when compared to well-studied systems including maize. Ultimately, across all systems, the impact of production on the linked processes of N leaching and volatilization must be quantitatively compared. To date, numerous studies have focused on N leaching in maize-based systems but few, if any, studies on N leaching potentials in switchgrass, Miscanthus, and sorghum biofuel systems have been done. Likewise, studies of management system impacts on N₂O emission are either relatively sparse (e.g. maize) or virtually non-existent (e.g. sorghum, Miscanthus). Poor understanding of N₂O emission represents a critical knowledge void as the radiative forcing of 1 kg of N₂O is equivalent to 296 kg CO₂; many have hypothesized that N₂O release from agro-biofuel production may negate any expected benefits in global warming reduction associated with fossil fuel displacement. While optimization of NUE is key to developing second-generation biofuels, the complex C-N interactions that drive N₂O emissions are not represented in LCA for most plant-soil ecosystems; there is a critical need for integrated research that captures air-water-plant-soil interactions in the context of biofuel production. This presentation will focus on key unknowns in nutrient use and fertilizer management for candidate biofuel cropping systems, and the potential for biotechnology solutions to improve nutrient use efficiency with a concomitant reduction in environmental impact.

Biosketch: Dr. Sylvie Brouder is a Professor of Agronomy in the Agronomy Department at Purdue University in West Lafayette, IN and Director of Purdue's Water Quality Field Station. She received her B.A. in Biology from Harvard University (1985) and her Ph. D. in Ecology from the Ecology Graduate Group at the University of California – Davis (1993). At Purdue, her area of specialization is crop mineral nutrition with an emphasis on rhizosphere ecology, crop ecology, water quality, and nutrient balances and losses in agro-ecosystems. She has expertise in analysis of large spatio-temporal datasets, application of mechanistic simulation models, and development/interpretation of soil and
tissue diagnostics. Dr. Brouder has research experience in cotton, rice, corn, soybean, and alfalfa production systems. Her appointment is split between research, extension education and on-campus teaching; at present, she also has an administrative appointment in Purdue’s College of Agriculture to promote all Agroecology programming. Dr. Brouder’s research interests include design/implementation of field and controlled environment experiments on nutrient budgets and plant-soil nutrient cycling processes and the preservation, curation and repurposing of the datasets from these experiments. She has focused on nitrogen, carbon and potassium, evaluating agricultural systems and management practices with respect to their practicality, ecological viability and sustainability, including influences on surface/groundwater quality and greenhouse gas emissions from agricultural soils. Current research at the WQFS is an interdisciplinary, team effort to understand the production and environmental implications of the U.S. biofuel agenda. The goal of this project is to develop a cropping system level analysis of the potential for Miscanthus, switchgrass, sorghum-based, maize-based and low-input native prairie production systems to provide renewable fuel while protecting natural resources. Key project collaborators are Dr. J. Volenec (physiology), Dr. R. Turco (soil microbiology), Dr. D.R. Smith (soil processes) and Dr. G. Ejeta (crop breeding and genetics). The overarching goal of the WQFS research portfolio is to underpin quantitative approaches or ecological accounting frameworks that can inform the development of policy to promote agricultural sustainability through valuation of the non-provisioning ecosystem services.

Establishing a feedstock supply chain for cellulosic ethanol in Tennessee

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Working with partners at Genera Energy LLC, the University of Tennessee’s (UT) Biofuels Initiative is developing a farm-to-fuel business plan supported by the State of Tennessee, the UT Institute of Agriculture, and Oak Ridge National Laboratory researchers. Tennessee has made an unparalleled commitment to lead the transition to an advanced biofuels economy with a commitment to support the development of a dedicated bioenergy crop supply chain and the construction of a 250,000 gallon (950,000 liter) per year demonstration cellulosic ethanol facility, which is now operational with partners at Genera Energy and Dupont Danisco Cellulosic Ethanol.

UT and Genera are establishing a dedicated energy crop (switchgrass) supply chain, to demonstrate and improve the technologies used to create cellulosic ethanol, reduce the costs of production, and ultimately commercialize the technology across the state. We work with local farmers to develop switchgrass production and provide one-on-one technical assistance through UT Extension and wide-ranging research related to all aspects of the feedstock supply chain. This research focuses on improving efficiencies and reducing costs in harvesting, storing, transporting, and preprocessing switchgrass feedstock. Approximately 5,100 acres (2,064
hectares) of switchgrass have been planted under the Biofuels Initiative's incentive program. The program has one of the largest plantings of switchgrass on private farms in the United States. Genera are also currently constructing the Tennessee Biomass Innovation Park to showcase processing, handling, storage, and conversion of a variety of cellulosic feedstocks.

The development of the bioenergy feedstock supply chain, from biomass to fuels and products, will have a significant impact on the energy future of the state and nation. The scale of work ongoing in Tennessee has provided significant opportunity to conduct research and development on agronomic production of biomass, transportation and logistics, storage, preprocessing, and conversion of biomass to fuels and products. This work will provide a viable path to a sustainable, biomass-based future for rural economies and the nation.

**Biosketch:** Dr. Jackson holds a faculty position at the University of Tennessee, serving as a Research Assistant Professor in the University's Center for Renewable Carbon. In his faculty role, he is focused on the research, development, and commercialization of sustainable feedstock supply chains for the emerging bioenergy industry. He works with a variety of feedstocks including perennial grasses, short-rotation woody crops, forest materials, and other agricultural crops and residues. A significant focus of effort for Dr. Jackson has been the $70 million University of Tennessee Biofuels Initiative. The program, funded by the state of Tennessee, seeks to integrate the switchgrass feedstock supply chain with bioenergy and bioproducts industries in the state. The program has led to the establishment of nearly 5,100 acres (2,064 hectares) of switchgrass and the construction of a demonstration-scale biorefinery. He has also served with the Southeastern Sun Grant Center, part of a nationwide bioenergy research program funded by the US Departments of Transportation, Energy, and Agriculture. Dr. Jackson has helped coordinate a regional grant program that has provided nearly $5 million for bioenergy research across the southeast. Dr. Jackson currently leads a $2.3 million project focused on switchgrass production that was competitively funded by the US Departments of Energy (DOE) and Agriculture. He is also part of another $4.9 million US DOE research project focused on high-tonnage logistics for switchgrass biomass.

As Genera Energy Vice President for Feedstock Operations, Dr. Jackson has worked to develop feedstock supply chains for bioenergy and bioproducts in Tennessee and the region. He has worked with university faculty, legislators, and farmers to spur the development of switchgrass as a bioenergy feedstock through the state’s Biofuels Initiative. Dr. Jackson works for sustainable, practical, and economical supply chain solutions.

Dr. Jackson received an undergraduate degree in Wildlife and Fisheries Science and his master’s degree in forest ecology and management from the University of Tennessee. He earned his doctoral degree in Natural Resources from the University of Tennessee as well.

**Evolution and divergence in the coding and promoter regions of the populus gene family encoding xyloglucan endotransglycosylase/hydrolases**

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Xyloglucan endotransglycosylase/hydrolases (XTHs) are believed to modify the cell wall structure by cleaving a xyloglucan polymer and transferring the newly generated, potentially reducing, terminal to another xyloglucan. We report here the detailed analysis of 37 *Populus trichocarpa* XTH genes/proteins in their divergence in both the coding and 5' promoter regions. Our results show that the *Populus* XTH genes have experienced whole-genome and local duplications, and pre-r and post-speciation divergence. Genome-wide and segmental duplications seem to be dominant in subfamily I and III, while tandem duplication seems to be the major mechanism for the subfamily II expansion, which also have higher average ratios of Ka/Ks compared to those in subfamily I and III. There was a general lack of organ-specific gene expression. In contrast, the expression patterns in subfamily II varied in response to various hormone and stress treatments, II-A being up-regulated and II-B down-regulated after 2-h of hormone and stress treatments. Expression for this subfamily was verified using the 1.5-kb PtXTH22 promoter that was fused with the GUS reporter gene and transformed into Arabidopsis. The PtXTH22 promoter contains AREF (auxin response element), EINL (ethylene insensitive 3 like factors), and BR (brassinosteroid response element) cis-elements. Histochemical GUS staining of transgenic Arabidopsis seedlings confirmed that the PtXTH22 promoter was up-regulated by several hormones. Overexpression of PtXTH22 resulted in resistance to ABA, mannitol, and some levels of resistance to stress.

**Biosketch:** Professor Cheng, received his BS from Nanjing and his MS and Ph.D from Cornell University in 1991. He was Assistant and Associate Professor at North Dakota State University from 1990-2001 with tenure, during which he also served as the Director of the Cellular and Molecular Biology Ph.D Program with over 30 faculty members from four colleagues. In 2001 he moved to University of Tennessee and now is the full professor in woody plant genomics, functional genomics and biotechnology. Since 2009, Dr. Cheng also serves a joint professor at Nanjing Agricultural University working on apple, grape and poplar genomics and biotechnology. He has published 55 refereed journal articles and holds and jointly holds five patents and three trademarks. Dr. Cheng also serves an Associate Editor for Plant Cell Tissue Organ Culture, HortSciences and Journal of Agricultural Biotechnology. Dr. Cheng also edited a special issue of Critical Review in Plant Sciences with Dr. Gerald Tuskan in the area of Populus Community Genomics, and special issue of Plant Cell Tissue Organ Culture with Dr. Schuyler Korban on Ploidy Manipulation in Genomics Era.

**Lignin modification improves saccharification efficiency of transgenic Populus tomentosa**

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Lignin is the major structural component of secondarily thickened cell walls of vascular plants, and has been recognized for its negative impact on forage digestibility, tree pulping properties, and more recently, cellulosic biofuel production. Over the last two decades, genetic manipulation of lignin biosynthesis has been well developed by engineering a wide variety of genes which are involved in monolignol biosynthesis in plants. Transgenic plants with modified lignin were shown to be improved on digestibility and pulping properties. The increasing interest in cellulosic biofuel production has become the new focus of lignin engineering. Understanding the performance of the lignin-modified poplar on conversion of lignocellulosic biomass to ethanol, especially on the process of saccharification would further hasten the application of lignin engineering on generating crops for biofuel production. In this study, the wood of transgenic poplar lines expressing the antisense transgenes of monolignol biosynthesis genes 4-coumarate: CoA ligase (4CL) or caffeoyl CoA 3-O-methyltransferase (CCoAOMT) were subjected to alkaline pretreatment and enzymatic hydrolysis. Analysis of lignin-modified poplars demonstrated significant increases in enzymatic hydrolysis efficiency. Increases of 22~48% in glucose released per gram dried, extractive-free biomass, and of 18~41% in enzymatic hydrolysis efficiency were observed in several independent transgenic lines. These improvements were associated with significant modification in lignin and cellulose content in five-year-old field-grown transgenic poplar and no observed phenotypic differences. These results imply that lignin modification can facilitate the process of saccharification for biofuel production in tree plants.

**Biosketch:** Dr. Hongzhi Wang is from Beijing Agro-Biotechnology Research Center of Beijing Academy of Agriculture and Forestry Sciences. She received doctoral degree in genetic from China Agricultural University in 2005. Her research focuses on lignin engineering in poplar for pulping properties and its application in biofuel production. Dr. Jianhua Wei is the director of Beijing Agro-Biotechnology Research Center of Beijing Academy of Agriculture and Forestry Sciences. He received doctoral degree from China Agricultural University in 1999. He has been working on poplar genetics in the University of Tennessee from 2004 to 2005. His research focuses on lignin modification in poplar for pulping properties and its application in biofuel.

**Proteomic analyses of the seed development of Jatropha curcas**

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*Jatropha curcas* L., a woody plant belonging to Euphorbiaceae family, is a promising economic plant due to the high oil content in seed. Combining with the paraffin section and physiological index, the proteomic analysis of seed development has been carried out. According to the seed length, fresh weight, dry weight and water content, the seed development of *J. curcas* have been divided into 3 different phase and 11 groups. In the early seed development, the nucleus of endosperm can be observed clearly base on the section. With the seed development, the cavity of endosperm cell were filled with a plenty of oil body when the seed was closed to maturity. G (Group) 2, G4, G6, G7, G8, G9, G10 and G11 were selected as protein extract materials. The 2-DE profiles of G2, 4, 6, 7 were compared with each other. 103 differential protein spots were detected and identified by Q-TOF/HDMS. The amount of metabolism and energy related proteins were 21% and 18%, respectively. 13 proteins were involved in network of carbon flux including glycolytic and oxidative pentose phosphate pathways. Most of these proteins were up-regulated in the early 4 experimental groups, which may suggest that carbon flux were synthesized in the early seed development and would be used for synthesis of fatty acid in the late development. The RuBisCO subunit was also detected in the early seed development, which may suggest the plastid have the potential for carbon fixation. Tubulin and actin related to the cell division covered the 4 experimental groups, and the 4th group has the highest abundance, which suggested that the cell division is in progress at the early seed development.

**Biosketch:** Hui Liu graduated in 2006 from Xiamen University with ecology major. She got the Master's degree in 2009 at Institute of Botany, the Chinese Academy of Sciences. Now, she is an assistant engineer as well as a doctoral candidate at Institute of Botany. Her research is focused on the seed development of *Jatropha curcas.*

Dr. 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.

**Cloning and functional characterization of fatty acid biosynthesis-related genes from *Jatropha curcas***

Pingzhi Wu, Guojiang Wu*
Jatropha curcas L. is a multi-use tree whose seeds may be useful as a feedstock for biodiesel. In order to determine the mechanisms involved in the biosynthesis of fatty acids in J. curcas seeds, we isolated several key enzymes involved in fatty acid desaturation, chain elongation and termination, and detected the expression patterns of these genes at various tissues and different seed development stages. Further, we address the question of which roles in fatty acid biosynthesis these enzymes play within the plant. We overexpressed two plastid-localized acyl-ACP thioesterases (JcFATA1 and JcFATB1) and two ER-localized fatty acid desaturases (JcFAD2 and JcFAD3) under a seed-specific promoter in Arabidopsis. Overexpression of JcFATA1 and JcFATB1 raised the saturated fatty acid contents in seeds. The contents of C18:0 in the seeds of JcFATA1 transformed plants increased by one or two fold. The contents of C16:0 of JcFATB1 transformed plants increased from around 9.0 mol% (in wild-type plants) to 30.4 mol%, and the highest was 40.9 mol%. Definitely, the unsaturated fatty acid contents in these two transgenic plants have decreased. The overexpression of ω-6 fatty acid desaturase (JcFAD2) enhanced the content of C18:2 obviously, and the content of C18:3 and C20:2 as well, however, the content of C18:1 reduced correspondingly. Besides, the overexpression of ω-3 fatty acid desaturase (JcFAD3) increased the content of C18:3 greatly, from around 15.8 mol% to 20.2 mol%, with the highest to 24.1 mol%, but the content of C18:2 reduced distinctly. Together, it can be seen that acyl-ACP thioesterases play an important role in controlling the lipid saturation and the carbon chain length, and the fatty acid desaturases (JcFAD2 and JcFAD3) can raise the content of unsaturated fatty acid in seeds apparently. In addition, the functional characterization of other fatty acid biosynthesis-related genes is ongoing.

Biosketch: Dr. Guojiang Wu is the Principle Investigator in the field of Sustainable Use of Plant and Gene Resource at South China Botanical Garden, Chinese Academy of Sciences (CAS). His major research interests are involved in plant molecular biology, mainly focused on mutant selection, functional genomics and gene transformation. Ongoing projects include energy plant exploitation, leguminous symbiotic nitrogen fixation, and rice genomic research work. Experience: 2004-Present: Professor, South China Botanical Garden, CAS; 1997-2004: Fellowship, Graduate School of the University of Tokyo; 1988-1990

Increasing seed yield in biofuel plant Jatropha curcas by manipulating floral development and sex expression
Jatropha curcas (jatropha), a tropical and subtropical shrub of the family Euphorbiaceae, is considered a potential biodiesel feedstock plant whose seeds contain up to 40% oil. As a monoecious perennial species, jatropha has few female flowers, which is one of the most important reasons for its poor seed yield. This study was undertaken to determine the effects of the plant growth regulator 6-benzyladenine (BA) on floral development and floral sex determination of J. curcas. Exogenous application of BA significantly increased total number of flowers per inflorescence, reaching a 3.6-fold increase (from 215 to 784) at 160 mg/L of BA. Furthermore, BA treatments induced bisexual flowers, which were not found in control inflorescence, and a substantial increase in the female-to-male flower ratio. Consequently, a 4.5-fold increase in fruit number and a 3.3-fold increase in final seed yield were observed in inflorescences treated with 160 mg/L of BA, which resulted from the much more female flowers and the new induced bisexual flowers in BA-treated inflorescences. This study indicates that the seed yield of J. curcas can be increased by the manipulation of the floral development and floral sex expression. Some of the possible transgenic approaches to engineer the metabolic pathways of cytokinins in jatropha inflorescence meristem will be discussed.

Biosketch: Dr. Zengfu Xu is a principal investigator, the head of the Laboratory of Molecular Breeding of Energy Plants, Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences (CAS), and the coordinator of the Energy Plant Research Consortium (EPRC) at XTBG. Dr. Xu's research interest is focused on the floral development and sex determination and oil (triacylglycerol) biosynthesis in the biodiesel feedstock plant Jatropha curcas (jatropha), a tropical and subtropical shrub of the family Euphorbiaceae. The long-term goal of Dr. Xu's laboratory is to improve jatropha seed yield and oil content through genomic and genetic engineering approaches combined with the techniques of biochemistry, molecular and cell biology, biotechnology, and traditional breeding, and to develop jatropha as a model plant for energy plant research.

Jatropha and sweet sorghum as an efficient bio-energy plants

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Both Jatropha and sweet sorghum have been regarded as major bio-energy plants. Jatropha oil from seeds can be processed to produce a high-quality biodiesel and soluble sugar from sorghum stem can be used to produce bio-ethanol. In Singapore, Jatropha R & D programs have been carried out by Temasek Life Sciences Laboratory (TLL) and JOil (S) Pte Ltd. The programs are focused on molecular improvement of Jatropha cultivars, genomics, genetics and molecular markers, photochemistry study, as well as the platform technology development. On the other hand, TLL and Institute of Botany (IOB), Chinese Academy of Sciences have set up the TLL-IOB joint lab. The major focus of the lab is on the sorghum functional genomics of sucrose metabolism. We have genome-widely identified multiple families of genes encoding various sucrose metabolism related enzymes. These enzymes include sucrose synthase, sucrose phosphate phosphatise, sucrose phosphate synthase and UDP-glucose pyrophosphorylase and so on. Most of these enzymes from sweet sorghum showed high homolog in their amino acids sequences when compared with grain sorghum. However, some of their genes exhibited significant variation in their exon splicing between sweet and grain sorghum. Our data also showed that some of sucrose metabolism related genes exhibited differences in their expression abundance and expression regulation between grain and sweet sorghum under sucrose treatment.

Biosketch: Dr. Srinivasan Ramachandran obtained his PhD in Biochemistry with Plant Molecular Biology as specialization from University of Idaho under the guidance of Prof David Oliver. He joined Prof Nam Hai Chua’s group at the Institute of Molecular and Cell Biology in 1993 and Institute of Molecular Agrobiology in 1996. He was the Principal Investigator of Rice Functional Genomics group at Institute of Molecular Agrobiology in 2001 and Temasek Life Sciences Laboratory since 2002. Currently, he is also a Principal Investigator of IOB-TLL Joint R&D Laboratory for Sweet Sorghum.
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Genetic diversity of switchgrass and its relative species in *Panicum* using SRAP and EST-SSR markers

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Switchgrass (*Panicum virgatum*) is a perennial C4 grass native to North America, which is used for soil conservation, forage production and more recently as a biomass crop for ethanol, fiber, electricity and heat production. *Panicum* is a cosmopolitan genus with approximately 500 species. The species conventionally assigned to the genus are widely distributed, from wet or dry woodlands, grasslands, shores, and marshes to disturbed areas and cultivated fields. So *Panicum* species has great potential for switchgrass resistance improvement. To study the genetic diversity and relationships between switchgrass and other species in *Panicum*, 91 accessions from USDA’s germplasm collection representing 22 species of *Panicum* from six continents and 22 countries were investigated by Sequence-related amplified polymorphism (SRAP) and expressed sequence tags-simple sequence repeat (EST-SSR) makers. Eight hundred and twenty-six markers from 28 pairs SRAP primers and 25 pairs EST-SSR Primers were used to differentiate between accessions of a bulk of 25 genotypes. The results showed as follows: (1) High genetic diversity was found in *Panicum* species (PPB=96.74%, I=0.463 based on SRAP data; PPB= 94.73%, I=0.424 based on EST-SSR data ), and genetic diversity in the *Panicum* species is more extensive than that in cultivated switchgrass; (2) Most genetic variation were present among the different species (70.02 % revealed by SRAP, 73.35% revealed by EST-SSR) and cluster analysis indicated that all the *Panicum* accessions could be distinguished by the SRAP or EST-SSR; (3) The different accessions in a species were clustered together, respectively, which had larger genetic similarities and closer relations. (4) Dendrogram faithfully reflected the phylogenetic relationships between *Panicum* species but did not indicate a possible domestication process of the cultivated switchgrass based on the geographical sources of the accessions. (5)*Panicum amarum* was the closest species to switchgrass; (6) SRAP methods were considered more efficient than EST-SSR for screening large numbers of *Panicum* accessions.
ISSR analysis of genetic diversity of the wide and escaped \textit{Medicago} L germplasm resources in Yunnan Province of China

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\textit{Medicago} L is a kind of excellent forages with high economical and ecological value. There are rich wide and escaped \textit{Medicago} L resources in Yunnan province. The aim of studying these \textit{Medicago} L resources in Yunnan is to figure out the genetic diversity of \textit{Medicago} L in various areas of Yunnan, and provide theoretic reference and technological reserve for preservation and utilization of \textit{Medicago} L resources in Yunnan.

30 wide and escaped \textit{Medicago} L germplasm resources in various areas of Yunnan were collected as the experimental materials. The genetic diversity of these resources was evaluated using ISSR markers. Data were analyzed by using POPGENE 1.31 and Mega2 tools.

The results showed that 18 primers were screened out from 56 ISSR primers totally to amplify the \textit{Medicago} L genome DNAs of the materials effectively. Among 243 sites amplified out totally, 216 were polymorphic and the ratio of polymorphic loci was 88.34\%, which proved that ISSR marker was an efficient tool for conducting genetic diversity studies in \textit{Medicago} L. The genetic distance of the 30 \textit{Medicago} L germplasm resources ranged from 0.05\textemdash0.78, and the average genetic distances between 1 and others were 0.7, which were greater. The UPGMA analysis of Nei’s genetic distance clustered the 30 wide and escaped \textit{Medicago} L germplasm resources to 6 groups when the genetic distance of 0.145 was used as the critical value. Results of cluster
analysis indicated that relationships among 30 wide and escaped *Medicago* L germplasm resources were related to their geographic distribution, and there were also some exceptions. *Medicago* L resources in Yunnan were rather rich. Relationships among 30 wide and escaped *Medicago* L resources in Yunnan were mainly related to their geographic distribution. Results indicated that the wide and escaped *Medicago* L resources in Yunnan had a wide genetic background.

**SRAP-based genetic diversity of *Jatropha curcas* grown in Hainan Province of China**

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Jatropha (*Jatropha curcas* L.) is consider as one of great potential biodiesel species, which have strong abilities of resistant to dry and infertile soil, rapid growth, easy propagation, high seed yield, higher oil content in kernel features. In the present study, we examined the genetic diversity and genetic structure of 42 provenances of *Jatropha curcas* including 418 individuals by using SRAP markers. Optimum system of SRAP was determined by orthogonal design. A total of 174 polymorphic loci were amplified and 423 alleles were detected using nineteen pairs of primers. Each primer amplified 18-27 DNA fragment, average 22; Percentage of polymorphism loci (PPL) was 23.12%. Average number of alleles (A) ranged 1.1749~1.7943, average effective number of alleles (Ae) ranged 1.0939~1.5414, Nei's gene diversity index (H) ranged 0.141~0.229, the value of Shannon's Information index (I) varied from 0.0569 to 0.3123. At the species level, the total genetic diversity (Ht) was 0.2020, and the genetic differentiation coefficient was 0.4708. Low gene flow (Nm = 0.5620) was detected among provenances. AMOVA analysis showed that genetic variation among the provenances accounted for 45.9% of the total. Principal coordinate analysis among individual indicated that 418 individual can be divided into three parts. Based on Nei's (1972) genetic distance, cluster analysis was carried out for various sources. The results of UPGMA indicated that 42 provenances could be divided into three types by a similar coefficient 0.122: Mexico's, India, Indonesia 2 and Vietnam clustered into one group; Indonesia 1 and 11 domestic provenances clustered into together; other 26 provenances clustered into one branch.
Mantel test revealed that genetic distance was not significantly related to geographical distance ($r = 0.0912, P = 0.827$). Composite above results showed that the majority of Jatropha in China's has close relationship, which is not related to the regional distribution. The genetic differentiation between provenances was small, genetic variation of *J. curcas* was derived from individuals within the provenances.

**Proteomic responses of Jerusalem artichoke to drought stress**

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Jerusalem artichoke (*Helianthus tuberosus* L.) is a perennial herb belonging to Asteraceae family. Its tubers are rich in fructose which can be used to produce ethanol. Due to its high resistance to adverse conditions such as drought, saline, Jerusalem artichoke is regard as a promising candidate crop for the manufacture of bioethanol. But the resistance differs a lot between Jerusalem artichoke cultivars, which prevent its further practice and application. In this study, we investigated proteomic responses of two different Jerusalem artichoke cultivars to polyethylene glycol (PEG) 6000 induced drought stress. Conclusions presented in this thesis can be summarized as follows:

Two-dimensional electrophoresis (2-DE) results showed 69 protein spots in cv. Xiuyan changed in abundance more than 1.5-fold after drought treatments, while 63 protein spots in cv. Yulin changed in abundance more than 1.5-fold. 46 proteins in cv. Xiuyan and 34 proteins in cv. Yulin were identified through LC-MS/MS and NCBI database searching. The identified proteins could be sorted into 6 functional categories, including photosynthesis, defense, signal transduction, carbon metabolism, energy and others. Further protein function analysis revealed the different protein regulation in two cultivars. Drought stress inhibited expression of some photosynthetic proteins in two cultivars. However, more proteins related to photosynthesis up-regulated in cv. Xiuyan after stress as compared with cv. Yulin. For example, expression of protein Rubisco large subunit in cv. Yulin was inhibited after stress, but it up-regulated in cv. Xiuyan. It may be explained that cv. Xiuyan increased expression of protein Rubisco large subunit to maintain a relatively high photosynthetic rate after drought stress. Drought stress also induced the expression of proteins related to defense in two cultivars. But more defense-related
proteins appeared in cv. Xiuyan, such as disease resistance-like protein, Peroxiredoxin-2F, lactoylglutathione lyase and so on. In addition, expression of proteins related to signal transduction, carbon metabolism and other functional categories also differed in two cultivars. Above all, cv. Xiuyan occurred more efficient protein regulations after drought stress, which resulted in the different drought resistance among two Jerusalem artichoke cultivars.

Identification of elite wild gene resources and germplasm enhancement on sweetpotato

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The behaviors of artificial selection and cross breeding result to genetic base becoming narrower and narrower, quick declining among alleles variation, which could cause some disease and pest spreading in a large scope and limit the development of crop breeding and genetics research about yield and quality. Wild relative species is referred to the progenitor or closer relationship to cultivated plants. They grow with the natural vegetation or weeds in field or in natural adversity circumstances. So they are becoming key genes carrier of disease-, pest-, abiotic stresses-resistance. According to incomplete statistics, more 5000 new varities belonged to 41 types of crops have been bred by utilizing wild gene resources, which are dedicated to disease-resistance, high-yield, excellent-quality and high-efficient agriculture. Ipomoea is a larger genus in the family Convolvulaceae, containing 600-700 species, which occupied one third of total species in the family. Among these wild species, many own the elite traits of disease-, pest-, drought- resistance, high-starch and salt-tolerance. However, sweetpotato cultivar I. batatas genetic base is narrow, and its origin and evolution are under hypothesis. To mine and use these elite genes from wild species and to widen sweetpotato genetic base, researchers did much jobs including the identification of morphology and resistance, and the studies of cytogenetics and molecular markers. Meanwhile, to transfer elien chromosomes or genes into cultivars, two approaches were mainly applied to interspecific hybridization. The first one is sexual cross, of which manual pollination combined with plant growth hormones treatment and young ovual rescue techniques. Second approach is cell fusion, in which somatic cell hybridization was done
by PEG methods. At present, more dozen of cross comparisions and interspecific hybrids from different wild parents were obtained, in which the hybrids using sexual hybridization were more than that using cell fusion technique. Moreover, the hybrids from the first approach own good characteristics of storage root-set. However, the hybrids from the second approach set poor fibre roots and their morphological characters are alike to their wild parents. By using sexual cross techniques, the tetraploid, pentaploid hybrids have been obtained. And by their backcrossing to cultivars, several larger populations of introgress lines (ILs) have been achieved to do genetic and molecular marker research. At last, the prospect and new thoughts of these novel germplasm applied to breeding and inheridity are put out by authors in future.

Efficient chloroplast transformation of *Phaeodactylum tricornutum* by electroporation

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Raw materials have become the largest bottleneck of biodiesel production. Marine microalgae have significant advantages for biodiesel production such as having abundant resource, high growth rate and high oil content. Microalga *Phaeodactylum tricornutum* has high fatty content up to 30%. Its biomasses have functional properties comparable to those of legume, and are a widely used forage species. Thus it is a promising candidate for the large-scale production of biodiesel. In plants, the plastid localization of fatty acid synthesis suggests that overexpression of key enzymes such as ACCase in the plastid could boost fatty acid production. Plastids are ideal hosts for expression of transgenes and have been used as bioreactors for production of various proteins. In this study, we report a high-efficiency chloroplast transformation system for *P. tricornutum*. This is the first chloroplast genetic transformation system for diatom and is based on the use of electroporation as a rather cheap and feasible approach. As an initial step towards expression of gene of interest, we have constructed the chloroplast transformation vector for *P. tricornutum*. In the vector, site of integration used is the transcriptionally active intergenic region between trnI and trnA genes, located in the IR regions of the chloroplast genome. We report the successful integration of the reporter genes *CAT* (coding for chloramphenicol acetyltransferase) and *GFP* (coding for green fluorescent protein)
integrating at this site in the chloroplast genome, which was confirmed by genomic DNA PCR and reverse transcriptase-PCR of stably transformed microalgae. The expression of CAT in transgenic microalgae confers resistance to the antibiotic chloramphenicol and enables transgenic microalgae to grow in the selective medium. The expressed GFP was found solely in chloroplasts. By using the improved electroporation procedure and the flanking sequences, we obtained about ten algal colonies every electroporation. Our results demonstrate the feasibility of foreign gene expression in chloroplast-transformed microalgae with important potential for producing biodiesel. Further study will be conducted on the expression of key enzyme such as acetyl-CoA carboxylase to improve the production of lipids.

Over-expression of \textit{JcDREB}, a putative AP2/EREBP domain-containing transcription factor gene in woody biodiesel plant \textit{Jatropha curcas}, enhances salt and freezing tolerance in transgenic \textit{Arabidopsis thalid}

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\textit{Jatropha curcas} is an all-purpose biodiesel plant and is widely distributed in topical and subtropical climates. It can grow well on poor quality land which is not qualified for crop cultivation. This is very important for relieving land, food and energy crisis. However, tropical and subtropical distribution limits the production of \textit{J. curcas} seed. So it is valuable to know the molecular mechanism of \textit{J. curcas} response to adverse abiotic environmental factors, especially freezing stress, in order to change the plant's characteristics. Until now there are just a few reports about \textit{J. curcas} molecular biology.

We have cloned and characterized a DNA binding proteins from an oil woody plant \textit{J. curcas}, designated \textit{JcDREB}. Sequence analysis and yeast one-hybrid assays show that \textit{JcDREB} protein can effectively function as a transcriptional factor of DREB protein family belonging to A-6 subgroup member. Expression patterns of \textit{JcDREB} showed that it was induced by cold, salt and drought stress, not by ABA. Over-expression of \textit{JcDREB} in transgenic Arabidopsis exhibited
enhanced salt and freezing stresses. Understanding the molecular mechanisms of *J. curcas* responses to environmental stresses, for example, high salinity, drought and low temperature, is crucial for improving their stress tolerance and productivity. Also this work provides more information about A-6 subgroup members of DREB subfamily.

**Comparison of the characteristics of fruit oil from five *Cinnamomum camphora* chemotypes**

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_Cinnamomum camphora_ is a large evergreen tree that grows up to 20–30 meters tall. It is native to southeast China, southern Japan and Indochina, where it is also cultivated for essential oil, timber production and landscaping. *Cinnamomum camphora* fruits contain more than 30% oil, and the relative contents of medium chain-length saturated fatty acids (capric acid and lauric acid) are more than 75%. The oil is a potential resource for light biodiesel. According to the differences of essential oil in leaves, *Cinnamomum camphora* is separated into five chemical variants called chemotypes, which are camphor, linalool, cineole, nerolidol or borneol. It can be distinguished by their odour. Fiber morphological feature, vessel morphological feature and wood basic density have no significant differences among five chemotypes. The aim of our work is to learn about the fruit oils, and to compare them among five chemotypes. *Cinnamomum camphora* fruit oil was extracted by Soxhlet method and acid value (AV), saponification value (SV) and iodine value (IV) were determined. Fatty acid methyl esters were prepared by acid catalysed esterification method and the profiles were analysis on a gas chromatography. The oil yields ranged from 34.32% for linalool chemotype to 39.54% for camphor chemotype. The results revealed that the chemotypes differed significantly with each other as far as total oil contents were concerned. The range of AVs (1.40-2.14 mg KOH/g) was low. It might make the process of refining a bit easier. The range of SVs from 264.89 to 270.45 mg KOH/g indicated that the oils held fatty acids with medium chain-length fatty acids. Indeed, the range of SVs for *Cinnamomum camphora* was quite similar to that of coconut oil (253-268 mg KOH/g). Low IVs (6.88-8.95) might be indicative of low level of unsaturated fatty acids in the oils. Ten fatty acids were identified in the oils. Capric and lauric acid were the two major fatty acids constituent,
ranging from 31.20 to 55.37% and 22.49 to 44.94% respectively. Interestingly, the content of lauric was higher than that of capric in camphor; however it was contrary in other four chemotypes. The saturated fatty acids were predominant in all oils as confirmed by the IV test. The total contents of unsaturated fatty acids ranged from 13.82 to 30.06%. Oleic acid was the main unsaturated fatty acid with 7.22-19.02% in the oils. The results might therefore offer a scientific basis for use of the *Cinnamomum camphora* fruits, both in the human diet and renewable energy.

**Analysis of oil content and fatty acid composition of the seed of wild oil plant in Northwest Sichuan Plateau of China**

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15 species of wild plants, *Descurainia sophia* L. (CRUCIFERAE), *Thlaspi arvense* L. (CRUCIFERAE), *Elsholtzia densa* Benth (LABIATAE), *Anisodus tanguticus* (Maxim.), Pascher (SOLANACEAE), *Carum carvi* L. (UMBELLIFERAE), etc., in Aba Prefecture in Sichuan Province of China were taken as the experimental material. SE and GC-MS was used for the determination of oil content and fatty acid composition of the seeds. The results showed that the oil contents of seeds were 47.44% for *Descurainia sophia* L., 34.7% for *Thlaspi arvense* L., 30.8% for *Elsholtzia densa* Benth, 18.5% for *Anisodus tanguticus* (Maxim.) Pascher and 31.4% for *Carum carvi* L., respectively. Fatty acid composition varies widely among different branches. The composition of CRUCIFERAE seed oil was particularly rich in oleic acid, linoleic acid, linolenic acid, 11-eicosenoic acid, palmitic acid, and oleic acid, linoleic acid and linolenic acid contents were relatively high. The main components of LABIATAE seed oil were linolenic acid, linoleic acid, which representing over 80% of the total fatty acids. The linoleic acid content of SOLANACEAE was more than 70%, while the oleic acid content was 57.5% in UMBELLIFERAE seed material. In short, seeds of wild plant materials tested were rich in oil. The fatty acid methyleate is the main component of biodiesel which can be used as an important future energy resource of plant development. Some reference value on the choice of materials for biodiesel was provided in this study.
Growth, physiology and nutrient concentration responses to water and nutrient stress of oil palm

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The research was conducted to detect the changes in growth, physiology and nutrient concentration in response to two watering regimes (well-watered and water-stress conditions) and to two nutrient regimes (with or without fertilization) of oil palm. Under stress conditions, some changes in growth and physiology were observed in oil palm for tolerance to the stresses, such as growth retard, dry matter allocation, decreased water content, reduced Chlorophyll a/b, leaf total N and P concentration and increased leaf relative conductivity and leaf total K concentration. These characteristics and related parameters were determined and the experiment result were listed as followed: (1) Fertilization promoted the growth of oil palm under well-watered conditions, while under water stress conditions its effects on growth was negative. The ratio of root/shoot was increased under water stress condition. (2) Relative water content and Chlorophyll a/b were decreased and leaf relative conductivity was increased under water and nutrient stress conditions. It is obvious that water stress had a far greater influence than nutrient stress on these parameters. (3) Fertilization decreased total N and P concentration while slightly increased total K under water-stressed conditions. These results showed that water × nutrient interaction had the greatest influence on changes in these traits of oil palm.

Lab-scale investigation of Laminaria japonica as a new energy crop candidate for simultaneous biogas and bioethanol production

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Bioenergy recovery from various kinds of energy crops has been extensively studied to replace the gradually depleted fossil fuels in order to solve our energy problem. However,
marine macro algae were not well explored as energy crop even though it holds several advantages compare with other biomass substrate. Therefore, in this study, a two-stage fermentation system was established under mesophilic condition to investigate the feasibility of Laminaria japonica (one brown algae species) for biogas and bioethanol production. As a result, in terms of bioenergy conversion, during the first stage (acidogenesis stage), 3.8% and 11.2% of total chemical oxygen demand (TCOD) were converted into hydrogen gas and ethanol, respectively, and during the second stage (methanogenesis stage), 46.1% of TCOD was converted into methane. Thus, compare with other energy crops, marine macro algae can be considered as a potential group to establish our future renewable energy system.

**Effect of salt on malondialdehyde and antioxidant enzymes in seedling roots of Jerusalem artichoke (Helianthus tuberosus L.)**

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Two cultivars of Jerusalem artichoke (Helianthus tuberosus L.) differing in genotype, Red skin (cv. R., salt tolerant but low-yield) and White skin (cv. W., salt-sensitive but high-yield), were used to investigate malondialdehyde (MDA) content and antioxidant enzyme activity changes in their roots under a hydroponic culture system with 250 mM NaCl. The results showed that MDA contents in roots of the two genotypes increased, but MDA content of cv. R. was higher than that of cv. W. Changes in all antioxidant enzymes in roots of both varieties exhibited a similar trend, namely increased initially and then decreased. However, there were still some differences existing between the two cultivars. In other words, activities of the other two antioxidant enzymes except catalase (CAT) and peroxidase (POD) in roots of cv. R. were less than controls at 48 h, while all others except ascorbate peroxidase (APX) in roots of cv. W. were greater than controls. The peak of superoxide dismutase (SOD) activity of cv. W. was observed to appear earlier than that of cv. R. CAT activity of cv. W. and was significantly greater than the value of cv. R. and the latter showed a moderate trend. POD activity of cv. R. obtained the maximum at 6 h, whereas the peak of cv. W. displayed at 24 h. APX activity of cv. R. declined more than that of cv. W. These results suggested that there was a lower efficiency of scavenging reactive oxygen species (ROS) in cv. R. roots. Concomitantly, salt stress caused more severe damage to roots of
cv. R. Antioxidant enzymes in roots were inadequate to elucidate salt-tolerance mechanisms of the whole plant.
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