

Energy Crop and Biotechnology for Biofuel Production

Selection of energy crops is the first priority for large-scale biofuel production in China. As a major topic, it was extensively discussed in the Second International Symposium on Bioenergy and Biotechnology, held from October 16–19th, 2010 in Huazhong Agricultural University (HZAU), Wuhan, China, with more than one hundred registered participants (Figure 1). In the opening ceremony, Liangcai Peng, Chair of the organization committee, briefly described bioenergy research progress in HZAU over the past two and a half years since the first symposium was held there in March 16–18th, 2008. He first reminded the participants that there is limited arable land available and a large population to serve in China, and then stressed the need to develop lignocellulosic ethanol using residues of major food crops (rice, maize and wheat) and entire biomass of C4 plants (miscanthus and sweet sorghum) that can be well grown in marginal lands. The energy crops were defined with a high yield of grain for food supply and readily decomposed cell walls for efficient biomass degradation and bioconversion into biofuels. Peng finally proposed three practicable approaches for energy crop discovery: natural germplasm resource collection, cell wall mutant selection and genetic manipulation. So far, his team has found a few promising materials, and predicted to partially utilize them as energy crops for an ethanol production pilot facility in cooperation with Auguan company.

Then, one of the two keynote speakers, Zhihong Xu, ex-president of Peking University, presented a general picture of transgenic plant biotechnologies and their potential applications towards energy crop improvement. Kenneth Keegstra, director of DOE Great Lakes Bioenergy Research Center USA, described their efforts to improve biofuel crops by modification of plant cell wall properties. The first is to modify lignin composition, the second is to increase the level of hexose-containing hemicelluloses, and the third is to understand glucomannan biosynthesis as a route to increased hexose content.

In the following session on energy crops, Haichun Jing from the Institute of Botany, Chinese Academy of Sciences (CAS), introduced sweet sorghum, a C4 model plant, which can be a dedicated biofuel crop through genome-based ge-

netic improvement. As a major food crop in China, rice was recommended by Guosheng Xie (HZAU) to be a potential energy crop. He presented more than one hundred rice mutants that showed distinct cell wall compositions and biomass degradation efficiencies in the mature straw. In terms of energy plants suitable for growing in marginal lands, Peng Zhang (Institute of Plant Physiology and Ecology, CAS) predicted a near future industrial application for starch ethanol using transgenic cassava and sweet potato; Guojiang Wu (South-China Botanical Garden, CAS, China) described their attempts towards genetic improvement of *Jatropha curcas* for biodiesel purpose; and Carl Kukkonen (Viaspac Inc., USA) introduced giant king grass (of an undisclosed plant genus) that can be harvested three times per year in the south of China.

In the session on biotechnology for biomass production, Herry Daniell (University of Central Florida, USA) and Jihong Liu Clarke (Bioforsk-Norwegian Institute for Agricultural and Environmental Research, Norway) pointed out that chloroplast genetic engineering is a particular and practicable method for generating transgenic plants at large-scale, and Mengzhu Lu (Chinese Academy of Forestry, China) proved that RNAi is an effective approach to reduce lignin content in poplar wood. Regarding biomass pretreatment and an integrated biorefinery approach, Arthur Ragauskas (Georgia Institute of Technology, USA) reported on his group's approach to elucidate crucial factors limiting conversion; Xiaowei Peng (Institute of Process Engineering, CAS, China) reported the potential towards a highly efficient degradation of lignocelluloses; and Yueqin Tang (Peking University, China) finally exhibited new facilities for a bioethanol pilot using sweet sorghum.

Another important topic was the genetic engineering of microorganisms for biomass conversion. The keynote speaker, Jindong Zhao, director for Institute of Hydrobiology CAS (China), presented a long-term perspective on cyanobacteria in biofuel research and application. Two speakers, Yinbo Qu (Shandong University, China) and Jiaxum Feng (Guangxi University, China), each described their methods for improvements of microorganism cellulase production and activity at various cultural conditions. In addition, Xiaoyu Zhang (Huazhong



Figure 1. Group photo from the 2nd International Symposium on Bioenergy and Biotechnology, October 16–19th, 2010 in Huazhong Agricultural University, Wuhan, China.

University of Science and Technology, China) and Jianshong Sun (Jiangsu University, China) respectively reported their efforts on white rot fungi used for lignin degradation and termite cellulolytic systems for lignocellulose bioconversion.

Plant cell wall biosynthesis was a major topic of the symposium. Vincent Bulone (Royal Institute of Technology, Sweden) presented the latest progress on cellulose biosynthesis in higher plants using biochemical, biophysical and proteomic approaches. Tony Bacic (University of Melbourne, Australia) reported a new finding that CslF and CslH gene families are both able to catalyze the assembly of (1,3;1,4)- β -D-glucans, which are major non-cellulosic polysaccharides in grasses. Two lectures focused on characterization of brittleness mutants for understanding of cell wall biosynthesis in grasses. Yihua Zhou (Institute of Genetics and Developmental Biology, CAS, China) identified several rice brittle mutants that reflect multiple pathways of cell wall biosynthesis, and Geoffrey Fincher (University of Adelaide, Australia) described two natural brittle stem mutants of barley. The mutants showed a reduced level of crystalline cellulose, which is caused by retro-element insertion into *HvCesA4* genes. These characteristic mutants provide the potential as energy crops for bioethanol production. In addition, Laigeng Li (Shanghai Institute for Biological Sciences, CAS, China) reported that the rice 4CL gene family may be crucial in controlling lignin metabolism, Yuhong Tang (The Samuel Roberts Noble Foundation, USA) identified novel transcripts involved in secondary cell wall formation in the vascular tissues of switchgrass using genomics tool, and Gongke Zhou (Qingdao Institute of Bioenergy and Bioprocess Technology, CAS, China) conducted a comprehensive analysis of NAC domain transcription factor family in *Populus trichocarpa*. Those fundamental findings provide clues for genetic manipulation of energy crops.

As an additional topic, three keynote speakers broadly discussed the political policy and economic strategy on developing energy crops and biofuels in China and beyond. Winston Ko introduced the research and education efforts on renewable energy in University of California, Davis, USA (UCD), and added the importance of engagement with government and business. Then, Minghua Zhang (UCD) analyzed potential impacts on biofuel production of global climate change, and suggested a solution by using residues of food crops and selecting drought-resistant energy plants. Finally, Shu Geng from Shenzhen Graduate School of Peking University highlighted the concept of 3L (low CO₂ emission, low energy consumption and low environmental pollution) as the challenge of our future life, and concluded that bioenergy, as one of the most important components, can provide the final solution for emission control and energy supply by continually developing 4G (four generations) biofuels: first generation, food crops for grain ethanol; second generation, residues of food crops/non-food plants for cellulosic ethanol; third generation, algal/cyanobacteria for ethanol and biodiesel and fourth generation, photosynthetic machinery into artificial systems for biohydrogen and others.

In the final plenary discussion, participants realized that the breadth and quality of biomass resources are key requirements for large-scale biofuel production in the future. Therefore, the selection of diverse energy crops is a promising solution, and miscanthus is considered as one of the best energy crops for marginal lands in China. This topic was discussed in-depth in the following Miscanthus Workshop.

In conjunction with the symposium, a special workshop was organized on one of the most important, emerging global energy crops, miscanthus. *Miscanthus* is a genus of C₄, rhizomatous perennial grasses native to southeast Asia, with

China being the most important center of diversity, given the broad geographic distribution of the species throughout the country. Several species of *Miscanthus*, including *M. sinensis*, *M. sacchariflorus*, *M. floridulus*, and *M. lutarioriparius*, are being studied as sources of varieties and allelic diversity as a biomass feedstock. The workshop was particularly timely, given the increased interest in miscanthus in the past 5 years, in the United States in particular, for an emerging biomass-based economy.

The workshop began with plenary lectures from Neal Gutter-son from Mendel Biotechnology, USA, and Joshua Yuan from Texas A&M University, USA, on commercial and technological perspectives. Neal provided a commercial perspective on miscanthus, focusing on a) the target geographies in the United States that are most promising for emergence of miscanthus as a biomass crop, and b) the development of a new seed system for miscanthus production that is critical to grower adoption of miscanthus as a crop. Current commercial varieties of miscanthus are of the species *M. x giganteus* (*Mxg*), with the major *Mxg* variety being a naturally occurring hybrid between *M. sinensis* and *M. sacchariflorus*. Mendel's approach to a seeded *Mxg* system was described. Joshua described some of the key tools that are applicable to miscanthus and other crops, as well as the analysis of the conversion of miscanthus into sugars for fermentation to biofuels.

Katrin Jakob, from Mendel Biotechnology described the application of molecular markers to genetic improvement of miscanthus. She has developed simple sequence repeat (SSR) markers to screen a collection of miscanthus originally developed by a German breeding company, along with some additional accessions from collaboration with Zili Yi and colleagues from the Hunan Agricultural University, China. These markers were largely based on SSR markers reported in sugarcane, approximately 50% of which have now been shown at Mendel to be conserved between *Miscanthus* and *Saccharum* species. Based on these and additional SSR markers, Mendel is collaborating with Andrew Paterson, from the University of Georgia, USA, to anchor the miscanthus genome against the well-characterized sorghum genome.

Cuixia Chen, from the University of Illinois, Urbana Champaign and the Energy Biosciences Institute, USA, (EBI), described her work to define the genome and transcriptome of *Mxg* and the related species, *M. sinensis* and *M. sacchariflorus*. The "gene space" of *Mxg* is estimated as ~150 Mb, based on the proportion of repetitive sequence in the large genome of *Mxg*, which is similar in size to maize. Therefore sequencing strategies are focusing on the gene space, with deep transcriptome sequencing being completed currently. The genome sequence is proving a formidable challenge given the triploid composition of *Mxg*, and the large amount of repetitive sequence. Nonetheless, 20x coverage of genome sequence

from 454 and Illumina machine sequencing is expected to be released by the end of 2010.

Qin Li, from the Biomass and BioEnergy Research Center, Huazhong University, China, described the characterization of cell wall composition in a selection of miscanthus natural accessions that were sourced from both the Wuhan Botanical Gardens, and a collection of the Hunan Agricultural University. Three species were sampled, *lutarioriparius*, *sacchariflorus* and *sinensis*, and significant differences were found in the species median and in the distribution of compositional values. The ease of deconstruction of cell walls into sugars, using hot water only, dilute acid or dilute base was also evaluated. Substantial variation was found in sugar release that merits further study to determine reproducibility of these effects under different growth conditions. The basis of such reproducible changes would form the basis for a genetic approach to the control of sugar yield, as linked to composition.

Junhua Peng, from the Wuhan Botanical Garden, CAS, China, described the WBG-CAS miscanthus collection, which was generated in the autumn of 2008. Over 600 accessions of *M. sinensis* were collected throughout China as rhizomes, to preserve their genetic identity, given the incompatible reproductive habit of most *Miscanthus* species. Not surprisingly, accessions from northern regions produce much less biomass generally than accessions from more southern locations. A broad range of flowering times was found in the collection. These resources are being used to produce new *Mxg* hybrids and *M. sinensis* cultivars.

Jianxiong Jiang, from Hunan Agricultural University, described an even broader and larger collection of miscanthus, which included 9 different species, and with over 1100 accessions, generated in collaboration with Mendel Biotechnology. Only 4 of these 9 species were found in significant numbers: *M. sinensis*, *M. sacchariflorus*, *M. lutarioriparius* and *M. floridulus*. The only commercial use of miscanthus in China was described in Hunan province, with a modest acreage of *M. lutarioriparius* used for paper production. Most of the natural accessions are diploid, with only a very few tetraploid or triploid. Significant variation was observed in flowering time, according to both species and geographical source. Northern *M. sacchariflorus* varieties, for example, flower generally much earlier than *M. sinensis* varieties found in southern China.

Finally, Bingsong Zheng, from Zhejiang A&F University, described initial efforts toward producing tissue culture-based methods for propagation or transformation of miscanthus, using embryogenic callus. In addition, work was described to test for salt tolerance of miscanthus seed, in order to identify promising varieties for more marginal production regions. In general, miscanthus was found to be sensitive to salt.

In conclusion, selection of energy crops offers promising solutions for large-scale biofuel production in China and

over the world. To reach the goal, the related biotechnology policies should be taken into account for legal guarantee and to ensure successful scientific work in the future. By presenting this meeting report in the JIPB Special Issue on Bioenergy Plants, we want to stress the importance of the matters discussed during the meeting. Finally, we would like to thank all participants and organizers for excellent contributions to the Symposium/Workshop. We are also indebted to JIPB for the publication of this report.

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