**HUAZHONG AGRICULTURAL UNIVERSITY**

**and**

**UNIVERSITY OF ARIZONA**

**FIRST BILATERAL SYMPOSIUM**

**ON CROP FUNCTIONAL GENOMICS**

# ABSTRACTS

**Wuhan, China**

**May 16 - 20, 2007**

# HUAZHONG AGRICULTURAL UNIVERSITY
# and
# UNIVERSITY OF ARIZONA

# FIRST BILATERAL SYMPOSIUM
# ON CROP FUNCTIONAL GENOMICS

It has recently been agreed that Huazhong Agricultural University and University of Arizona establish a joint laboratory (called Virtual Center) on crop functional genomics to strengthen the relationship of collaborative research and personnel exchanges. Both universities have traditional strengths in research programs and training curricula in genetics, genomics and crop improvement. The UA has developed strong programs in the area of genomics, epigenetics, proteomics of crop plants and HZAU in genetics, molecular biology and biotechnology with applications to crop improvement.

A bilateral symposium will be held during May 16-20, 2007 in Wuhan on the campus of Huazhong Agricultural University, to exchange information on research and development in functional genomics, epigenetics, abiotic stress, reproductive development and evolution, disease resistance of crop plants, and to discuss issues regarding further collaborative research activities and personnel exchanges of faculties and students between the two universities.

**Co-organized by:**
Huazhong Agriculture University
University of Arizona

**Co-Chairs:**
Dr. Qifa Zhang (Huazhong Agricultural University)
Dr. Rod A Wing (University of Arizona)

**Hosted by:** National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University

**Sponsors:** Supported by the 111 Project (高等学校学科创新引智计划资助，项目编号：B07041) and the National Natural Science Foundation of China

# AGENDA

THE FIRST HZAU-UA BILATERAL SYMPOSIUM

ON CROP FUNCTIONAL GENOMICS

May 16-20, 2007

## May 16, 2007 Arrival

**14:30 – 18:00 Campus and Lab Visits**

## May 17, 2007

**8:30 – 9:00 Opening Remarks (Chair: *Qifa Zhang*)**

***Duanpin Zhang*** President, HZAU

**9:00 – 9:30 Collaborative Agreement Signature**

(Remarks by Qifa Zhang and Rod Wing)

**9:30 – 12:10 Session 1 (Chair: *Meizhong Luo*)**

***Talks are 20 min each plus 5 min for discussion***

- **Functional and Comparative Genomic**

***9:30 – 9:50*** ***Vicki L. Chandler*** Overview of the BIO5

***9:50 – 10:15*** ***Jian Xu*** Polar transport sufficient for stable developmentally instructive auxin maximum and gradient

***10:15 – 10:40*** ***Rod Wing*** The *Oryza* map alignment project: a new resource for comparative genome studies within *Oryza*

**10:40 – 11:55 Tea Break**

***10:55 – 11:20*** ***Kede Liu*** Development of microsatellite markers for genetic mapping of important agronomic traits in *Brassica napus*

***11:20 – 11:45*** ***Marc Orbach*** A genomics approach to understanding pathogenicity of the rice blast fungus, *Magnaporthe oryzae*

***11:45 – 12:10*** ***Yonglian Zheng*** Maize genomics research in Huazhong Agricultural University

**12:10 Lunch**

**13:30 – 18:00 Session 2 (Chair: *Yongmin Zhou*)**

- **Genetic and Genomic Resource Creation; Abiotic and Biotic Stress and Signaling**

***13:30 – 13:55*** ***Bin Yi*** Fine mapping and physical delimitation of the RGMS gene *Bnms1* to a 21-kb DNA segment

***13:55 – 14:20*** ***Jinling Meng*** Genome-wide analysis of epigenetic effects on QTLs controlling important agronomical traits in rapeseeds

***14:20 – 14:45*** ***Ravishankar Palanivelu*** Right on target: Multiple signals guide pollen tubes to ovules to affect fertilization

***14:45 – 15:10*** ***Meizhong Luo*** A resource creation platform for structural and functional genomics

***15:10 – 15:30*** ***Shiping Wang*** Research programs in rice disease resistance group

**15:30 – 16:00 Tea Break**

***16:00 – 16:25*** ***Zhongguo Xiong*** Explore pathogenomics and molecular plant-virus interaction to improve crop resistance to diseases

***16:25 – 16:50*** ***Fangsen Xu*** Studies on Boron and Phosphorus use efficiency in *Brassica napus*

***16:50 – 17:15*** ***Lizhong Xiong*** Molecular basis and genetic improvement of drought resistance of rice

***17:15 – 17:40*** ***Elizabeth Vierling*** Genes and gene networks controlling tolerance to high temperature

***17:40 – 18:05*** ***Yunjiang Cheng*** Citrus genetic improvement and breeding in China

**18:10 Dinner**

**20:00 Cocktail Party**

## May 18, 2007

**8:30 - 12:05 Session 3 (Chair: *Elizabeth Vierling*)**

- **Gene Regulation and Expression; Biotechnology**

***8:30 – 8:55*** ***Vicki L. Chandler*** Mechanisms of gene regulation and gene silencing

***8:55 – 9:20*** ***Shuangxia Jin*** Transgenic cotton production: Focus on efficient methods development

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| ***9:20 – 9:45*** | ***David R. Gang*** | Applying "Omics"-based technology to medicinal plant research |
| ***9:45 – 10:10*** | ***Yuqing He*** | Gene pyramiding to improve hybrid rice by molecular marker-aided selection |

**10:10 – 10:25 Tea Break**

| | | |
|---|---|---|
| ***10:25 – 10:50*** | ***David W. Galbraith*** | High-throughput methods for analyzing gene expression in higher plants |
| ***10:50 – 11:15*** | ***Rod Wing*** | Presentations of the UA scientists that are not attending this meeting |
| ***11:15 – 11:40*** | ***Yongjun Lin*** | The production of insect-resistant *indica* rice by introduction of synthetic *Bt* genes |
| ***11:40 – 12:05*** | ***Qifa Zhang*** | Progresses of rice functional genomics research in China |

**12:05 – 13:30 Lunch**

**13:30 Departure for the Three Gorges**

## May 19, 2007

**19:30 – 22:00 Collaboration Discussion on the Boat**

# Progresses of Rice Functional Genomics Research in China

***Qifa Zhang***

National Key Laboratory of Crop Genetic Improvement and National Center of Plant Gene Research (Wuhan), Huazhong Agricultural University, Wuhan 430070, China

China initiated a national key project on rice functional genomics in 2002 with the long-term goal to identify the functions of all the genes in the rice genome together with the joint efforts in the international community. The first phase (2002-2005) of the Chinese Rice Functional Genomics Program is composed of three components: (1) development of technological platforms, (2) functional genomics of agriculturally important traits and, (3) molecular cloning of functional genes. The platforms aimed at enabling high throughput analyses and effective characterization of gene functions, which consist of three major parts: generation and characterization of a large mutant library by T-DNA insertion; expression profiling of the predicted exons and ESTs of the entire genome; and isolation of full length cDNAs of an indica line. The traits targeted for functional genomic studies in this program include grain quality, yield, stress tolerance, disease and insect resistance, and nutrient efficiency. Totally 270000 independent transformants have been generated for the mutant library which are now being screened for mutations for an array of traits. Over 20000 flanking sequences have been isolated, analysis of the flanking sequences identified a number of interesting features of the T-DNA insertions in the rice genome. A large number of mutants has now been targeted for gene isolation. Three classes of DNA chips (cDNA, oligo-nucleotides and tiling array) have been developed for profiling gene expression; data have been collected from more than 30 tissues covering the whole life cycle of the rice plants. Map-based cloning has been applied to isolate genes of agronomic importance. More than 30 genes have been cloned using this approach including genes for yield, grain quality, fertility restoration, disease resistance and salt tolerance. The next phase (2006-2010) will continue essentially along the same lines. In addition, a systematic approach has been designed to phenotypically characterize all the mutants available for chromosomes 3 and 4.

# The Oryza Map Alignment Project: A New Resource for Comparative Genome Studies within *Oryza*

***Rod A. Wing***
Arizona Genomics Institute and BIO Institute, University of Arizona, Tucson, AZ

With the completion of a finished genome sequence we must now functionally characterize the rice genome by a variety of methods including comparative genomic analysis between cereal species and within the genus *Oryza*. *Oryza* contains 2 cultivated and 22 wild species that represent 10 distinct genome types. The wild species, in particular, contain an essentially untapped reservoir of agriculturally important genes that must be harnessed if we are to maintain a safe and secure food supply for the 21st century. OMAP was established two years ago to generate a comprehensive set of genomics resources to investigate genome evolution and enhance positional cloning efforts in the genus *Oryza*. To date we have generated: A) 12 high quality BAC libraries that encompass the 10 genome types of *Oryza*; B) ~1000 Mb of BAC end sequence from these libraries; and C) SNaPshot fingerprint databases for all 12 libraries. All of these resources are publicly available through the AGI BAC/EST Resource Center, GenBank or at www.OMAP.org. The fingerprints and end sequences (BES) have been combined to develop 12 phase I physical maps.Eight of these physical maps, O. nivara [AA], O.rufipogon [AA], *O. glaberrima* [AA], *O. punctata* [BB], *O. officinalis* [CC], *O. australiensis* [EE], *brachyantha* [FF], and *O. minuta* [BBCC] have been heavily manually edited (HME) and aligned to the reference rice genome sequence. These alignments have revealed a large array of genome rearrangements relative to the IRGSP reference sequence and have allowed us to begin draw a more complete picture of *Oryza* genome evolution. In this talk I will present the current status of OMAP and discuss recent analysis of the HME maps, a global analysis of structural variation among the AA genome species, comparative sequence analysis of select loci across *Oryza*.

# Maize Genomics Research in Huazhong Agricultural University

***Hailin Xiao***

Natinal Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, 430070, China

At present, the maize genomics research in Huazhong Agricultural University mainly focuses on several agricultural traits, such as S type cytoplasmic male sterility (CMS-S), high lysine mutant in opaque endosperm, submergence tolerance, salt tolerance and head smut disease resistant. In research of these traits, both structural and functional genomics methods were used, such as genomic mapping, molecular marker-assisted selection, cDNA microarray, suppression subtractive hybridization (SSH), etc. To construct the functional research platform in maize, we established one library of Mu-mediated mutants and another of single segment substitution lines (SSSLs).

In the research of maize CMS-S, we got to know that the sterility is a result of programmed cell death (PCD) process happened at the tetraploid stage. The results of cDNA microarray also detected several PCD associated genes expressed highly in the sterile microspores. However, corresponding PCD inhibitors were up-regulated in the fertility-restored microspores. In previous report, *orf355-orf77* was identified as CMS-S associated fragment in maize (Zabala *et al*., 1997). In the fertility restored pollen, the amount of *orf355-orf77* transcripts was greatly reduced. By comparison of the *orf355-orf77* transcripts between sterile and restored pollen, it was found that the 5′ stem-loop structure was cleaved in the latter. It is proposed that the deletion of the 5' stem-loop facilitates the degradation of *orf355-orf77* transcripts, which further leads to the decrease of their amount (Xiao *et al*., 2006). We have been mapping the restorer gene *Rf3* since 1995, and recently it was mapped at a locus 0.9 CM to a marker (Zhang *et al*., 2006). The fine mapping of *Rf3* is undergoing.

A mutant of high lysine in endosperm, namely *o16*, was found and this gene was mapped at either 2.2 or 3.0 CM from UMC1141 on the long arm of chromosome 8 in two $F_{2:3}$ populations, respectively. In the population crossed with *o16* line and another *o2* line, the average lysine content of the F3 *o2o2o16o16* families was approximately 30% higher than that of the F3 *o2o2* and *o16o16* families, respectively (Yang *et al*., 2005). The potential application of the *o16* mutant may be combining it with the *o2* mutant, thus obtaining a line with much higher lysine content.

Submergence tolerance is a useful trait for maize breeding in South China. Through SSH and cDNA microarray between the treated and untreated HZ32 (tolerant) and Mo17 (sensitive), more than 100 genes were found to be differently expressed in the early response of submergence tolerance, among which several potential tolerant genes were

analyzed, such as Zm-bRLZ, ZmZf and Cyp51. Besides, several OTLs with high contribution were located based on the traits of plant height, shoot dry weight, root height and root dry weight of waterlogged seedlings at the second leaf stage (unpublished).

Salt soil greatly reduces the corn production in large coastal regions of China. Our lab launched on the salt tolerance research in 2003 and identified the NC286 as tolerant material and Huangzaosi as sensitive material. Based on the analysis of SSH and cDNA microarray, 292 differently expressed clones were sequenced, among which 166 sequences represented tentative unique genes (TUGs). Besides, 117 out of 252 SSR primers showed polymorphism between NC286 and Huangzaosi. Based on the population of these two parental varieties, the salt tolerance associated QTLs were mapped primarily at 9.03, 9.07 and 5.07 Bin (unpublished).

Head smut is one of the three major corn diseases in China, and mainly spreads in the spring corn regions. Generally the head smut is infected into maize plants during the budding stage and its final sympton occurs until the tasseling stage. We developped a PCR method to judge whether one plant is infected by *Sporisorium reilianum* during the seedling stage. With the SSH and cDNA microarray analysis of the infected and uninfected Mo17 (resistant) or Huangzaosi (sensitive) plants, respectively, 83 TUGs were found to be differently expressed during the resistant pathways.

For further functional genomic research in maize, one library of Mu-mediated mutants and another of single segment substitution lines (SSSLs) were constructed. The mutants and SSSLs will facilitate a shortcut in isolation and map cloning of functional genes.

Reference


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2. Xiao H L, Zhang F D, Zheng Y L. The 5' stem-loop and its role in mRNA stability in maize S cytoplasmic male sterility. *Plant J*, 2006, 47: 864–872
3. Zhang Z F, Wang Y, Zheng Y L. AFLP and PCR-based markers linked to *Rf3*, a fertility restorer gene for S cytoplasmic male sterility in maize. *Mol Genet Genomics*, 2006, 276: 162-169
4. Yang W, Zheng Y, Zheng W, Feng R. Molecular genetic mapping of a high-lysine mutant gene (*opaque*-16) and the double recessive effect with opaque-2 in maize. *Molecular Breeding*, 2005, 15: 257-269

# A Genomics Approach to Understanding Pathogenicity of the Rice Blast Fungus, *Magnaporthe oryzae*

***Marc Orbach***
Department of Plant Sciences, University of Arizona, Tucson, AZ

Towards the goal of identifying the genes necessary for pathogenicity in *Magnaporthe oryzae*, we have used transformation-mediated insertional mutagenesis to create a collection of >55,000 random DNA insertion lines of *M. oryzae* strain 70-15. Both Ca/PEG transformation of protoplasts and *Agrobacterium tumefaciens*-mediated transformation were used to produce the insertion lines. All strains were put through a series of phenotypic screens to identify genes involved in growth rate, conidiation, pigmentation, auxotrophy, and pathogenicity. Pathogenicity mutants were identified both by infection assays on whole plants, and through an in vitro screen for appressorium development. More than 250 mutants were identified in the screens, and recovery of the tagged genes is underway.

Recovery of a set of 256 random DNA insertions from T-DNA insertion lines has indicated that insertions are distributed throughout the genome although there may be some bias in insertion sites. Most insertions produced small deletions at the point of insertion with 90% being less than 500 nt. A limited number of insertions have been recovered and analyzed from pathogenicity mutants. Unlike in plant T-DNA insertional mutagenesis, it appears that most T-DNA insertions in *M. oryzae* are responsible for the mutant phenotype that is observed. Complementation of insertion lines with wild-type gene copies allows this assessment. Among the mutants analyzed, we have identified new genes in gene families that are involved in pathogenicity in *M. oryzae*, including an ABC transporter and a cyclophilin. In addition we have identified a number of new putative pathogenicity genes in *M. oryzae* that will be presented.

# Comparative Analysis of A-, C- and AC-genome of *Brassica* Species

***Chunyu Zhang[1], Ruiyuan Li[1], S.R. Choi[2], Yan Long[1], Congcong Jiang[1], Xingxing Wang[1], Yongbiao Ma[1], Y.P. Lim[2], Jinling Meng[1]***

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To understand genes underneath QTL in an un-sequenced genome such as *Brassica napus* (AC), one of facing troubles is how to develop polymorphic molecular markers within the QTL interval region. Comparative analysis, especially with genome sequenced relatives, is regarded as an effective strategy to solve this problem since their organization is highly conserved at the microstructure levels. The genome relationship among *B. napus*, *B. rapa* (A) and *B. oleracea* (C) are well defined, and are the closest crop relatives to the model plant Arabidopsis. Former results showed that there are about 21 segments of the genome of Arabidopsis, representing almost its entirety, could be duplicated and rearranged to generate the structure of the *B. napus*. Additionally, more and more sequences data from the recently diverged *Brassica* A and C genomes will available due to the Genome Sequencing Project. Appropriate applications of bioinformatics and comparative approaches will enable the integration of the microstructures across species and facilitate QTL analysis. Meanwhile, genome rearrangement among the A-, C- and AC-genome will be revealed. In this study, comparative analysis of A- and AC-genome will be reported; the strategy for comparison of C- and AC-genome will be discussed.

# Unique Chromosome Behavior and Genetic Control in *Brassica* × *Orychophragmus* Wide Hybrids: a Review

***Zaiyun Li, Xianhong Ge***

National Key Lab of Crop Genetic Improvement, National Center of Crop Molecular Breeding, National Center of Oil Crop Improvement (Wuhan), College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China. Correspondence to Z. Y. Li. E-mail: lizaiyun@mail.hzau.edu.cn

Researchers recognized early that chromosome behavior, as other morphological characters, is under genetic control and gave some cytogenetical examples, such as the homoeologous chromosome pairing in wheat. In the intergeneric sexual hybrids between cultivated *Brassica* species and another crucifer *Orychophragmus violaceus,* the phenomenon of parental genome separation was found under genetic control during mitosis and meiosis. The cytogenetics of these hybrids was species-specific for *Brassica* parents. The different chromosome behavior of hybrids with three *Brassica* diploids *(B. rapa, B. nigra and B. oleracea)* might contribute to the different cytology of hybrids with three tetraploids *(B. napus, B. juncea* and *B. carinata)*. The finding that genome-specific retention or loss of chromosomes in hybrids of *O. violaceus* with *B. carinata* and synthetic *Brassica* hexaploids (2n=54, AABBCC) is likely related to nucleolar dominance gives new insight into the molecular mechanisms regarding the cytology in these hybrids. It is proposed that the preferential expressions of genes for centromeric proteins from one parent (such as the well presented centromeric histone H3) are related with chromosome stability in wide hybrids and nucleolar dominance is beneficial to the production of centromere-specific proteins of the rRNAs-donor parent and to the stability of its chromosomes.

**Keywords:** *Brassica* species, *Orychophragmus violaceus*, Intergeneric hybrids, Cytogenetics, Genome separation, Chromosome elimination.

# Genome-Wide Analysis of Epigenetic Effects on QTLs Controlling Important Agronomical Traits in Rapeseeds

***Jinling Meng, Jiaqin Shi, Yan Long, Mingqin Shao, Wei Xia, Chunyu Zhang***
National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China

DNA methylation has been revealed to be a common phenomenon in genome of Arabidopsis and its effects on regulation of gene expression via chromatin remodeling has been demonstrated widely in plants. To understand epigenetic effects on traits of rapeseeds genomically, QTLs involving 11 traits of *Brassica napus* under 7 environments were subjected to analyze their relationships with DNA methylation loci on a genetic map constructed with the QTL mapping population. One hundred and eighty-eight methylation-sensitive loci (M-loci) distributing on all of 19 linkage groups were detected on the 820--marked map. The density of M-loci varied very much with linkage groups, from every 5cM to 52cM for one M-locus with 17 cM on average. Thirty-three M-loci showed remarkable correlation ($p<0.001$) with the 11 traits and the traits of seed development as well the trait of flowering time were severely effected by DNA methylation. The nature of the methylation-related QTL will be further studied.

# Right on Target: Multiple Signals Guide Pollen Tubes to Ovules to Affect Fertilization

***Ravi Palanivelu***
Dept. of Plant Sciences, University of Arizona, Tucson, Arizona

Pollen tubes navigate past a variety of pistil cells, most likely with the help of adhesive, attractive and repulsive cues, to deliver sperm to egg contained within ovules. To characterize the dynamics of pollen tube migration and identify the guidance signals, we developed an in vitro assay in the model plant Arabidopsis thaliana to study pollen tube guidance to ovules.

Using this assay, we characterized four signaling events that regulate pollen tube guidance in A. thaliana:

1) Pollen tube capacitance: Pollen tubes acquire ovule-targeting competence only after growing on the female tissues,
2) Pollen tube attraction: Unfertilized A. thaliana ovules emit diffusible, developmentally regulated, species-specific attractants,
3) Pollen tube repulsion: Ovules penetrated by pollen tubes rapidly release diffusible repellents to prevent additional tubes from entering them,
4) Pollen tube reception: Synergid cell death initiates after the pollen tube arrives at the female gametophyte but before pollen tube discharge.

Our current efforts are focused on isolating and characterizing the signals that control each of the above mentioned four signaling events by employing a variety of traditional approaches (genetics, cell biology, biochemistry) in combination with global approaches (proteomics, microarray and metabolomics). A overview of our efforts in characterizing each of these signaling steps will be presented.

References:

Palanivelu, R., and Preuss, D. (2006). Distinct short-range ovule signals attract or repel Arabidopsis thaliana pollen tubes in vitro. BMC Plant Biology 6:7.
(Time-lapse image files used in this study can be accessed at this website: http://www.ag.arizona.edu/research/ravilab/lab%20images&movies%20page.html).
Palanivelu, R., Brass, L., Edlund, A., and Preuss, D. (2003). Pollen tube growth and guidance is regulated by POP2, an Arabidopsis gene that controls GABA levels. Cell 114:47-59.

# Rice Genomic Resources for Functional Studies

***Meizhong Luo***

*Haiyan Lin[1], Nick Sisneros[2], Hye Ran Kim[2], So-jeong Lee[2], Dave Kudrna[2], Jose Luis Goicoechea[2], Wolfgang Golser[2], Elizabeth Ashley[2], Kristi Collura[2], Jennifer Currie[2], Yeisoo Yu[2], Bin Han[3], Qifa Zhang[1], Rod Wing[2],* ***Meizhong Luo[1]*** *(mzluo@mail.hzau.edu.cn)*

[1]College of Life Sciences and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China;

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BAC libraries and physical maps are essential for map-based cloning of functional genes and QTL and for functional genomics studies. The *indica* rice variety 93-11 is a parent of the super-hybrid rice LYP9 widely grown in China and a recipient of gene introgression from *japonica* rice. The *indica* rice varieties Minghui 63 (MH63) and Zhenshan 97 (ZS97) are the parents of Shanyou 63, another hybrid rice also widely grown in China, and the parents for genetic mapping. Many genes and QTL have been genetically mapped using these two varieties. The *japonica* rice variety Zhonghua 11 (ZH11) is the host for an ever-enlarging T-DNA insertional mutant library and an up-coming EMS mutant library. Except for MH63, for which a BAC library was made about 10 years ago, no other BAC libraries and physical maps were available for the above elite rice varieties, although the 93-11 genome was sequenced through the whole genome shotgun (WGS) strategy.

We constructed BAC libraries for 93-11, MH63, ZS97 and ZH11 at an about 10X genome coverage each with average insert sizes of 143 kb, 125 kb, 117 kb and 127 kb respectively. The whole 93-11 BAC library and half of the MH63 and ZH11 BAC libraries were both end sequenced and fingerprinted, and half of the ZS97 BAC library was end sequenced. We also end sequenced 9312 BAC clones of the previous MH63 BAC library. A physical map of 93-11 is being constructed using the BAC end sequences and fingerprints. This physical map will be used to verify the 93-11 WGS assembly and close gaps. The end sequences of the ZS97 BAC library and the previous MH63 BAC library were mapped to the nipponbare pseudo-molecules and already provided positions and BAC clones for cloning of four gene loci. Other analyses are under way. BAC library construction for the other parent of the super-hybrid rice LYP9 is also in progress.

# Fine Mapping and Physical Delimitation of the RGMS Gene *Bnms1* to a 21-kb DNA Segment

***Bin Yi, Yuning Chen, Shaolin Lei, Chaozhi Ma, Jinxing Tu, Tingdong Fu***

National Key Laboratory of Crop Genetic Improvement, National Sub-center of Rapeseed Improvement in Wuhan, Huazhong Agricultural University, Wuhan 430070, China

A recessive genic male sterility (RGMS) system, S45 AB, has been developed from spontaneous mutation in *Brassica napus* canola variety Oro, and is being used for hybrid cultivar development in China. The male sterility of S45 was controlled by two duplicated recessive genes, named as *Bnms1* and *Bnms2*. To better understand the molecular basis underlying recessive genic male sterility, a map-based cloning strategy has been employed to isolate *Bnms1*. Seven *Bnms1*-linked AFLP markers were developed and four of them were converted to sequence-characterized amplified region (SCAR) marker. A high-resolution genetic map was developed using a NIL population consisting of 4,132 individuals, in which the recessive allele was homozygous at the second locus. Recombination suppression was observed in the vicinity of *Bnms1*. Three molecular markers tightly linked to *Bnms1* were identified and used to screen a BAC library. A contig spanning the *Bnms1* locus was constructed and physical mapping delimited *Bnms1* to a 21-kb DNA segment within a single BAC clone. These results provide the essential information for the final isolation of this important gene in rapeseed microsporogenesis.

**Keywords:** Recessive genic male-sterility · *Brassica napus* · S45 AB · Genetic mapping · Physical mapping · BAC contig

# Explore Pathogenomics and Molecular Plant-Virus interaction to Improve Crop Resistance to Diseases

***Zhongguo Xiong***
Department of Plant Sciences, University of Arizona

A key to improving crop resistance to viruses is to better understand viral genetic determinants for pathogenesis and the molecular interplays between host and viruses. Toward this goal, several complementary research projects are undertaken in my laboratory. A pathogenomics project taking advantages of resequencing microarray and 454 sequencing technologies is aimed to sequence genomes of a large number of *Citrus tristeza virus* isolates, to build a comprehensive genomic sequence database, and to identify viral genetic determinants for pathogenesis and other biological properties by database mining. During pathogenesis and viral infection, crucial viral proteins often play multiple important roles, and consequently are attractive targets for molecular control strategies. We are currently characterizing molecular mechanisms and dissecting functional motifs of the capsid protein encode by a small RNA virus in viral cell-to-cell movement, long distant transport, and RNAi suppression. This project is made possible by an experimental platform that provides functional complementation of cell-to-cell movement through transgenic expression of a heterologous movement protein in plants, and functional complementation of RNAi suppression though chimeric expression of an unrelated RNAi suppressor in the viral genome. RNAi is a natural, sequence-specific defense response of plants against viruses and can be manipulated to improve crop resistance to viral infections. In addition to engineer RNAi-based immunity to viruses in plants, we are also investigating molecular mechanisms of virus-encoded proteins in RNAi suppression and the impact of RNAi suppressors on the stability of genetically engineered, RNAi-based resistance. Future research in my laboratory will explore possibilities of manipulating plant genes for durable and broad spectrum resistance. Viruses have limited coding capacity due to their compact genomes and thus absolutely require functions provided by a large number of plant genes to complete their life cycle in the host. These plant genes are likely conserved and important in normal cellular processes; however they could be surgically modified to block their interactions with viral proteins while maintaining normal cellular functions, therefore opening possibilities to engineer a broad spectrum of resistance to a large number of viruses in future.

# Germplasm Identification and QTLs Mapping for Born and Phosphorus efficiency in *Brassica napus*

***FS Xu, L Shi, YLHan, CY Zeng, H Zhao, YH Wang, JL Meng***

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China

Rapeseed (*Brassica napus*) is one of main oil crops in China, which planting area reached 8 million hectares, and the output of the seed yield is 12 million tons each year now. In the aspect of nutrient demand, rapeseed is one of the most sensitive crops to boron (B) and phosphorous (P) deficiency. There are large-scale B- and P-deficient cultivated lands in the planting district of rapeseed. Genetic improvements of the B and P nutrition traits are an important approach for the sustainable production of rapeseed in China.

210 cultivars of *Brassica napus* were screened by a two-step methods, the first pace was that all cultivars were grown with pot culture for 35 d under low B (0.10 mg B/kg) and high B (1.0 mg B/kg), then biomass was harvested and the ratio of dry weight under low B to high B was calculated. The cultivars, which the ratio was less than 0.50 or more than 0.90, were selected to go into the second pace. The candidated cultivars were grown under another two B levels, low B (0.25 mg B/kg) and high B (1.0 mg B/kg) up to harvest seed yield. The cultivar, which B efficient coefficient (the ratio of seed yield under low B to high B) was more than 0.90, was defined as B–efficient (resistant to B-deficiency) cultivar, if its ratio was less than 0.10, it belonged to B-inefficient (sensitive to B-deficiency) cultivar. By the two-pace screening, we confirmed eight B-efficient cultivars and two B-inefficient cultivars. Stronger B uptake and reutilization ability of B-efficient cultivars at B deficiency might be two main physiological mechanisms of B efficiency. One major QTL, *BE1*, for seed yield under low B representing B efficiency was mapped on the N2 linkage group of *B. napus* in both a $F_2$ population and a $F_{2:3}$ population, and another more than 30 QTLs for B content, B accumulation, root length, shoot and root dry weight at seedling stage under low B was detected in a double haploid population derived from Tapidor, a B-inefficient cultivar, and Ningyou 7, a B-efficient cultivar. Now near iso-genic lines in rapeseed were developed for fine mapping the *BE1*. The *BE1* was mapped on chromosome 1 of *Arabidopsis* in an interval of 6.4 cM by comparative mapping between *Brassica* and *Arabidopsis*, which was overlapped with a QTL interval controlling *Arabidopsis* B efficiency.

A P-efficient cultivar “97081” and a P-inefficient cultivar “97009” were obtained by screening 243 rapeseed cultivars at seedling stage and whole growth period. A genetic linkage map with 23 linkage groups was constructed using SSR and AFLP markers in a $F_{2:3}$ population derived from a cross between “97081” and “97009” , and three QTLs , *DSY*1, *DSY*2, *DSY*3, which significant related to seed yield at P deficiency and one QTL of P

efficiency coefficient (PECM ) were detected in genome-wide QTL analyses with WinCarter2.0 software.

**Key word:** *Brassica napus,* boron, phosphorus, nutrient efficiency, germplasm identification, QTL mapping

# Molecular Basis and Genetic Improvement of Drought Resistance of Rice

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Drought frequently occurs in late stages of growth and development of rice and causes significant yield loss. We have integrated approaches including germplasm screening, genetic analysis, functional genomics, and transgenic to identify the genetic and molecular basis of drought resistance of rice. Progresses will be presented in the meeting in the following outline. (1) Germplasm screening. More than 10000 T-DNA insertion mutant families and about 200 Condensed Core Collection of rice germplasms have been screened under drought conditions at vegetative or reproductive stages. A few mutants that were more sensitive or tolerant to drought stress than the wild type have been identified. A few drought resistant germplasms were added to the backcross breeding programs. (2) Genetic analysis of drought tolerance. Using a plant-wise drought treatment of a recombinant inbred line population, drought tolerance was separated from drought avoidance, and QTL mapping results suggested drought tolerance and drought avoidance had distinct genetic basis. More than 20 near isogenic lines of drought resistance QTLs are under construction. (3) Expression profiling analysis of rice under drought stress. Using cDNA microarray and DNA chip technologies, more than 1000 genes responsive to drought at reproductive stage were identified. A significant proportion of genes showed differential responsiveness to drought stress between upland and irrigated rice. (4) Functional analysis of genes conferring drought resistance in rice. More than 50 drought inducible genes with unknown function have been overexpressed or knockdown in rice cultivar Zhonghua 11 for genetic effect testing. A few regulatory genes were functionally characterized. (5) Molecular breeding of drought resistance of rice. A few function-known genes were transformed into Zhonghua 11 to identify genes conferring drought resistance at reproductive stage. Marker-assisted selection has been used to introduce alleles of drought resistance QTLs from upland rice IRAT109 to irrigated rice Zhenshan 97.

# Genes and Gene Networks Controlling Tolerance to High Temperature

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The long term goals of our research are to understand the genetic pathways and biochemical components that confer tolerance to high temperature in plants. We are using forward and reverse genetics, gene microarray methods and biochemistry to define components involved in the response to high temperatures in the model plant *Arabidopsis thaliana*. Using forward genetics, we isolated mutants unable to acclimate to heat stress (*hot* mutants). The first mutant uncovered encoded the molecular chaperone Hsp101 (*hot1*). We are continuing genetics and biochemistry to define the mechanism and targets of cytosolic Hsp101 and of related proteins in chloroplasts. Major recent efforts concern the *hot5* locus, which encodes S-nitrosoglutathione reductase (GSNOR), which metabolizes GSNO, a nitric oxide (NO) adduct of glutathione. GSNO is proposed to act as a reservoir for NO *in vivo*; thus, an understanding of GSNO metabolism is important for understanding NO signaling. GSNOR null mutants also have reduced fertility, implicating NO in successful pollination. To identify heat stress signaling components we have screened both EMS and T-DNA mutagenized transgenic plants expressing an Hsp101promoter::Luciferase transgene. Reverse genetics and biochemistry are being used to investigate the family of small HSPs, including proteins localized to the cytosol (for which we have a crystal structure), the chloroplast, mitochondrion and peroxisome. We have also used whole-genome microarrays to investigate the changes in transcript levels that occur during the acquisition of thermotolerance in 7-10 day old Arabidopsis plants. Gene cluster analysis revealed that in addition to molecular chaperone functions, degradation of damaged proteins, protection of post-transcriptional processes, prevention of programmed cell death, and reduction of both cellular metabolism and functions associated with disease resistance are all unique to thermotolerant plants. We would like to expand these studies to investigate related chaperone mutants and other stress tolerance mutants in rice with a special emphasis on field performance.

# An Initial Step toward Understanding the Molecular Mechanism of Dominant Genic Male Sterility in Rapeseed

***G S Yang, D F Hong, P W Liu***

National Key Laboratory of Crop Genetic Improvement, huazhong Agricultural University, Wuhan China, 430070

Male sterility (MS) can be generally classified into cytoplasmic MS (CMS) and genic MS (GMS), both of which are important approaches for crop heterosis utilization. In order to understand the molecular mechanism of some spontaneous male sterility mutant in rapeseed, we have spread our research work on the fine mapping of two male sterility genes from a dominant GMS and a recessive GMS, respectively.

Rs1046AB is a dominant GMS two-type line in rapeseed and the sterility of it was conditioned by the interaction of a male sterility gene (*Ms*) and its allelic restorer gene (*Mf*). After integrating the results of three different mapping populations, we have positioned the *Ms* gene in a genetic region of 4.6 cM by nine molecular markers. Among them, one cosegregated with the *Ms* gene, the other two closest markers bracketed it with a genetic distance of 0.1 cM and 1.0 cM, respectively. Moreover, comparison of these markers' distribution in genetic linkage map and their homologous loci in *Arabidopsis* revealed that there was a collinearity relation between the region flanking *M*s and *Arabidopsis* homologues, though it was likely disrupted by some chromosome inversion and translocation events.

9012AB is another kind of GMS mutant, the sterility of which is controlled by two pairs of recessive duplicate sterile genes (designated *Bnms3* and *Bnms4*) interacting with one pair of a recessive epistatic inhibitor gene (*esp*). Using a NIL population combining AFLP primer screening, we have identified many markers tightly linked with the $BnMs_3$ gene. Some of these markers were converted to SCAR markers and then analyzed in a larger NIL population of 9,600 individuals. Three SCAR markers have been identified to co-segregate with the target gene. By southern blotting and PCR assay, we have identified a *Brassica napus* BAC clone in which the three SCAR markers are all comprised. However, since no recombinant event has occurred in this region, we can't further identify the exact region containing the candidate gene in the BAC clone.

# Biographical Sketch

**Vicki L. Chandler**

Dept. of Plant Sciences and Molecular & Cellular Biology & Director of The BIO5 Institute, University of Arizona

**PROFESSIONAL PREPARATION**

U.C. Berkeley, CA, Undergraduate Research with Professor Randy Schekman, Dept. of Biochemistry, Cum Laude & major honors, B.A., 1978

Cold Spring Harbor Laboratory, CSH, NY, Summer Undergrad. Res. Prg., Dr. R. Gestland, 1977

U.C. San Francisco, CA, Ph.D., Biochemistry Dept. of Biochemistry & Biophysics, 1983

Stanford University, Stanford, CA, Postdoctoral Fellow, Dept. of Biological Sciences, 1983-85

**APPOINTMENTS**

2006-present Member, Arizona Cancer Center, University of Arizona, Tucson

2005-present Weiler Endowed Chair in Agriculture and Life Sciences, Univ. Arizona

2004-present Director, BIO5 Institute, University of Arizona, Tucson (Co-Director 2002-2004)

2003-present Regents' Professor, University of Arizona, Tucson

1997-present Professor, Dept. of Plant Sciences, University of Arizona, Tucson

1998-present Professor, Molecular Cellular Biology Dept., Univ. of Arizona, Tucson

1998-present Member, Interdisciplinary Program in Genetics, Univ. of Arizona, Tucson

1985-1997 Assistant, Associate and Full Professor of Biology; Member, Institute of Molecular Biology, University of Oregon, Eugene, OR

**PUBLICATIONS:**

**Five Publications Most Closely Related (total 80):**

Alleman, M., Sidorenko, L., McGinnis, K., Seshadri, V., Dorweiler, J.E., White, J., Sikkink, K., and Chandler, V.L. 2006. An RNA-dependent RNA polymerase is required for paramutation in maize. *Nature* **442**(7100): 295-298.

McGinnis, K.M., Springer, C., Lin, Y., Carey, C.C., and Chandler, V. 2006. Transcriptionally silenced transgenes in maize are activated by three mutations defective in paramutation. *Genetics* **173**(3): 1637-1647.

Lisch, D., Carey, C.C., Dorweiler, J.E., and Chandler, V.L. 2002. A mutation that prevents paramutation in maize also reverses *Mutator* transposon methylation and silencing. *PNAS* **99**(9): 6130-6135.

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with paramutation. *Genes Dev* **16**(15): 1906-1918.

Dorweiler, J.E., Carey, C.C., Kubo, K.M., Hollick, J.B., Kermicle, J.L., and Chandler, V.L. 2000. *Mediator of paramutation1* is required for establishment and maintenance of paramutation at multiple maize loci. *Plant Cell* **12**(11): 2101-2118.

**Five Other Significant Publications:**

Chandler, V.L. 2007. Paramutation: From Maize to Mice. Cell **128**(4), 641-645.

Selinger, D.A., Lisch, D., and Chandler, V.L. 1998. The maize regulatory gene *B-Peru* contains a DNA rearrangement that specifies tissue-specific expression through both positive and negative promoter elements. *Genetics* **149**(2): 1125-1138.

Goff, S.A., Cone, K.C., and Chandler, V.L. 1992. Functional analysis of the transcriptional activator encoded by the maize *B* gene: evidence for a direct functional interaction between two classes of regulatory proteins. *Genes Dev* **6**(5): 864-875.

Radicella, J.P., Brown, D., Tolar, L.A., and Chandler, V.L. 1992. Allelic diversity of the maize *B* regulatory gene: different leader and promoter sequences of two *B* alleles determine distinct tissue specificities of anthocyanin production. *Genes Dev* **6**(11): 2152-2164.

Goff, S.A., Klein, T.M., Roth, B.A., Fromm, M.E., Cone, K.C., Radicella, J.P., and Chandler, V.L. 1990. Transactivation of anthocyanin biosynthetic genes following transfer of *B* regulatory genes into maize tissues. *Embo J* **9**(8): 2517-2522.

**SELECTED HONORS AND AWARDS:**

| | |
|---|---|
| 2005 | Recipient, NIH Director's Pioneer Award |
| 2005 | Weiler Endowed Chair for Excellence in Agriculture & Life Sciences, U. Az.. |
| 2005 | Fellow, American Association for the Advancement of Science (AAAS) |
| 2003 | Regents' Professor, University of Arizona |
| 2002 | Elected to the National Academy of Sciences, USA |
| 2001 | College of Agriculture and Life Sciences, Faculty Researcher of the Year Award |
| 1991 | National Science Foundation, Faculty Awards for Women Scientists & Engineers |
| 1988 | Searle Scholar |
| 1985-1990 | Presidential Young Investigator Award |
| 1983-1985 | National Science Foundation Plant Biology Postdoctoral Fellowship |

**SYNERGISTIC ACTIVITIES:**

My research focuses on the mechanisms of gene regulation and gene silencing. Results from my lab have had significant implications, not only for the field of plant genetics but also for understanding human disease. I have trained many undergraduates, including underrepresented minorities. To show the diversity of my lab, 2006 personnel were 3 high school students (1 Asian), two of whom continued in the lab as undergraduate assistants; 1 high school teacher summer intern; 12 undergraduates (7 female; 1 Hispanic); 2 summer

undergraduate interns (1 female from small college in the east and 1 Navajo) and two rotation students (both female). Also in the lab were three graduate students (2 female, 1 Brazilian, 1 Asian); 3 postdocs (1 female, 2 Polish, 1 Hispanic); 3 research scientists (2 female) and 1 research professor. In addition, there are three female Hispanic technicians and one Asian technician. For the past five summers I have hosted high school and community college biology teachers in my laboratory, helping them develop curricula using corn in the classroom. One of the teachers is from the Navajo reservation in which 98% of his students are Native American. He later communicated to me that the use of corn, very important in Native American religion and culture, has stimulated great interest among his students.

I have served on numerous federal competitive grant panels for NSF, NIH, DOE, and USDA. In 2001 I was appointed to the NSF Biological Directorate Advisory Committee and have testified before federal congressional committees. I served on the Board of Reviewing Editors for *Science* and am on the editorial boards *Genetics, Plant Physiology*, and the *Annual Review of Plant Biology*. I have served on the Board of Directors for Genetics and the International Society of Plant Molecular Biology, on the Board of Trustees for the Gordon Research Conferences, and as President of the American Society of Plant Biologists. Service to the state of Arizona includes membership on the Board of Directors of the Bioindustry Organization of Southern Arizona, the Arizona Bioscience Roadmap Steering Committee of the Flinn Foundation, and the National Advisory Board of the C-Path Institute. I am an appointed Commissioner on the State of Arizona Commerce and Economic Development Commission. In 2004 I was appointed Director of the BIO5 Institute, an interdisciplinary program drawing from agriculture, basic science, pharmacy, engineering and medicine at the University of Arizona.

**Graduate Advisor:** Graduate: Keith R. Yamamoto, Univ. CA, San Francisco; Postdoctoral advisor: Virginia Walbot, Stanford, CA. Total graduate students trained: 15; total postdoctoral fellows: 20

# Developing Excellent Transformation Systems in Cotton

***Shuangxia Jin, Xianlong Zhang (xlzhang@mail.hzau.edu.cn)***
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High efficiency of transformation procedure relies on the high regeneability of the explants used. Some Coker varieties have been reported to have the highest regeneration potential compared to other varieties. However, plant regeneration in Chinese cultivar of Upland cotton YZ-1 through somatic embryogenesis was found to show predominant ability of somatic embryogenesis over Coker lines in our lab, which got high ratio of somatic embryogenesis within two months.

Embryogenic callus as perfect explants for transformation has been successful on many species for its small size, condensed cytoplasm and rapidly dividing cell cultures. More recently, we developed a reliable and high-efficiency system of transforming embryogenic callus (EC) *via Agrobacterium tumefaciens* in cotton. An overall scheme for producing transgenic cotton is presented, through which an average transformation rate of 15% was obtained.

A successful transformation program relies on the number of survival plants in soil that can be obtained. An efficient grafting system for recovering plants derived from somatic embryogenesis following *Agrobacterium* infection and kanamycin selection was developed. Various aspects of *in vitro* grafting were examined in efforts to improve the efficiency of transformant recovery. Over 90 % successful grafting ratio could be obtained under optimal conditions, which represented a significant improvement over currently available methods for recovery of cotton plantlet from somatic embryogenesis after transformation.

*GFP* as a visual marker gene was used for detection early evens of transformation in our laboratory. The results showed that transient expression of *GFP* can happen on the epidermal cells and primary vascular structure, but only a few stable transformation evens existed on epidermal cells. Transient expression and stable expression of report gene were two correlative but different things. To improve the transient expression and reduce the damage to the explants could indeed improve the transformation efficiency. Most of the stable transformation evens happened on the primary vascular structure. Morphological character and growth of transgenic and non-transgenic calli were different. Within two months after transformation, transgenic calli grew slowly, but they would rapidly proliferate two months later. Most of the kanamycin-resistant calli were composed of transgenic and non-transgenic calli. Characters of growth of four genotypes after transformation *via Agrobacterium* were different. Somatic embryogenesis of transgenic calli relied on high concentration of hormone.

Based on the high efficient transformation and plant recovery system described above, many foreign genes have been transferred into cotton genome. For example, *ipt*, *barnase*, *bar*, *Chitinase* and *Glucanase* , *Cry1C, Cry2A* and Cry9C genes have been successfully transferred *via Agrobacterium*-meditated transformation in our laboratory.

# Isolation and Analyses of Genes Preferentially Expressed during Sea-island Cotton (*Gossypium barbadense* L.) Fiber Development by Microarray and Quantitative Real-time PCR

***Lili Tu, Xianlong Zhang* Shaoguang Liang, Di-qiu Liu, Longfu Zhu, Fanchang Zeng, Yichun Nie, Xiaoping Guo, Fenglin Deng, Jiafu Tan, Li Xu***

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Sea-island cotton (*Gossypium barbadense* L.) is one of the most valuable cotton species due to its silkiness, luster, long staples, and high strength, but its fiber development mechanism has not been surveyed comprehensively. We constructed a normalized fiber cDNA library (from –2 to 25 dpa) of *G. barbadense* cv. Pima 3-79 (the genetic standard line) by saturation hybridization with genomic DNA. We screened Pima 3-79 fiber RNA from five developmental stages using a cDNA array including 9126 plasmids randomly selected from the library, and we selected and sequenced 929 clones that had different signal intensities between any two stages. The 887 high-quality expressed sequence tags obtained were assembled into 645 consensus sequences (582 singletons and 63 contigs), of which 455 were assigned to functional categories using gene ontology. Almost 50% of binned genes belonged to metabolism functional categories. Based on subarray analysis of the 887 high-quality expressed sequence tags with 0-, 5-, 10-, 15-, and 20- dpa RNA of Pima 3-79 fibers and a mixture of RNA of nonfiber tissues, seven types of expression profiles were elucidated. Our results showed that phytohormones play an important role in the fiber development. We also compared the different development stages' expression profiles between *G. barbadense* and *Gossypium hirsutum* L. The expression profiles of these cultivated tetraploid species may help us to decipher the differences in quality and yield between the two.

We also confirmed the data from microarray in detail by quantitative real-time PCR analysis. For accurate and reliable gene expression results, validation of internal control genes is required. We assessed the gene expression of 7 frequently used housekeeping genes, including *18S rRNA*, *Histone3*, *UBQ7*, *Actin*, *Cyclophilin, Gbpolyubiquitin-1*and *Gbpolyubiquitin-2*, in a diverse set of 22 cotton samples (9 fiber samples at different development stages and 13 nonfiber tissues). The expression of *Histone3*, *UBQ7* and *Gbpolyubiquitin-1* was most stable for the nonfiber tissues series. No gene found stable for fiber developmental series and the relative quantification was not efficient with the 7 commonly reported 'housekeeping' genes. And the relative absolute quantification should be an efficient and convenient method for the fiber developmental series.

# A Gene Regulatory Network for Totipotency Cytogenetic Reprogramming during Cotton Somatic Embryogenesis

***Fanchang Zeng, Xianlong Zhang*(xlzhang@mail.hzau.edu.cn), Lisong Hu, Jinglin Cao, Longfu Zhu, Xiaoping Guo and Yichun Nie***

National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, China

Somatic embryogenesis (SE) is the developmental reprogramming of somatic cells toward the embryogenesis pathway, which is a notable illustration of cell totipotency. SE is a unique developmental pathway and has been viewed as a potential model system for the study of the basic mechanisms of development reprogramming among the higher eukaryotic organirms, nevertheless it attracts wide interest in understanding totipotency of cells. Despite of the very extensive tissue culture research, we are far away from understanding the key molecular events leading to SE. It is a very difficult task to extract the experimental findings and provide a comprehensive view. In our investigation, transcriptome profiling and hierarchical clustering of SE-associated transcript tags during the whole somatic embryogenesis process in cotton was comprehensively analyzed. A broad repertoire of SE genes was identified, which are an important resource for understanding the genetic interactions underlying SE signaling and regulation. The complexity of somatic embryogenesis trancriptome suggests that large numbers of molecules are involved in plant cell totipotency. In order to understand the concerted mechanism involving multiple cellular pathways and the relationships of molecular events unfolding during this important reprogramming process from functional genomics assays readout to high level biological data interpretation, we then used bioinformatics and system biology strategy to constructed a molecular interaction network draft based on transcriptionally regulated SE-related genes, which is suggested to represent the complexity of events and processes in the SE system. Here, a complex molecular system was unraveled by SE association network. Highly specific discovery of SE transcriptionally regulated genes combined with logical SE association networks predict various cellular pathways and genes involved in SE. By analyzing the draft network, the processes of cell death and cell proliferation were revealed to be significant components of SE as the extremely noteworthy SE association biomarkers. In addition, the cross talks and dynamic balance in the interactions among all hormones (Auxin, Ethylene, Brassinolide, Gibberellins, Cytochalasin and ABA) which regulate (positive or negative) the distinct processes of cell death and cell proliferation suggest they are pivotal in switching cell fate during the developmental plasticity of SE through coordinated interactions with many developmental signaling pathways. This report represents a comprehensive analysis of the SE association network in plants. Then we assessed the

overall predictive value and the elements this draft molecular network by examining its fit to published SE data based on experiments by independent laboratories. We convincingly show that important features of this association network are in good accordance with the published data. We evaluated the predictions for a considerable number of SE association biomarkers which have been independently validated in cultured plant cells. The result shows that our approach is feasible and that the predictions are successful significantly, although the accurate estimate about SE association network required more accumulation of published documents. The approach presented here is scalable and can be extended to include additional data types. In particular, with more information of studies and pathway database becoming available, including those in database and documents, we expect this effective system approach to be further promote the development of biology association network and adapted to various targeted gene network in the future. Furthermore, we are now performing robustness analyses to testify the roles and interaction relation of SE association biomarkers in SE association regulatory networks in series of our experiments using gene RNAi, overexpress and interaction relation assay. The aim of our investigation was to confirm the crucial molecular components involved in SE association networks and understand the precise mechanism controlling the differentiation of somatic cells and the detailed steps by which these genes direct SE. It ultimately will provide deeper insight into understanding the enigmatic reprogramming of cells in higher plants. The present work is the first successful attempt in describing a putative interaction regulation network that can underly the SE. This work could provide a useful test for modeling of a plant systems network and may have merit as a study presenting an advanced technology application in plant biology due to its biological and economical importance.

# Linkage Map Construction and QTL Mapping in Cotton

***Zhongxu Lin, Xianlong Zhang*, Daohua He, Yichun Nie, Xiaoping Guo***

National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, China

Cotton is the leading natural fiber crop and a major oilseed crop in the world, and therefore has been concerned by cotton breeders and farmers. Cotton breeders are trying to improve cotton traits by genetic methods and great succeeds have been achieved. However, most of the cotton traits are quantitative traits; improvements by traditional methods have reached a platform. In order to make an obvious progress, new strategies are necessary in cotton breeding. Our lab is devoted in cotton genetics and breeding, a set of molecular linkage maps have been constructed and some QTLs for important traits have been mapped, which will be helpful in marker assisted breeding.

An interspecific $F_2$ population consisted of 69 plants, which was developed from the cross between *Gossypium hirsutum,* cv. Handan208 (characterized as high fiber yield) and *G. hirsutum,* cv. Pima90 (characterized as excellent fiber quality), was genotyped with SSR, RAPD, SRAP and REMAP markers. Over 2210 primers (combinations) were used to survey the polymorphism between the parents, and revealed 1394 polymorphic loci, 1029 loci were arranged into 26 linkage groups. The linkage map covered 5472.3 cM with an average distance of 5.32 cM between two markers. QTL affecting fiber yield and quality traits in 69 $F_{2:3}$ family were analyzed and yielded 52 QTLs: four for lint index, eight for seed index, ten for lint yield, four for seed cotton yield, nine for number of seed per boll, three for fiber strength, five for fiber length, and eigth for micronaire value.

In order to fine mapping these QTLs, an advanced-generation population of this cross was developed by single seed descent to F6 generation. The introgression population was genotyped using SSR, and the Pima 90 allele frequency at each locus was 0.21 in mean; in individual introgressed lines, the percentage and length of chromatin segment introgressed from Pima 90 were 21.4% and totally 947.8 cM in mean, respectively. Phenotyping and phenotypic distribution indicated a trend of regression of individual lines to Handan 208. Significant loci influencing fiber quality were detected by one-way ANOVA ($P$<0.005): 5 markers for fiber length, 4 markers for uniformity, 2 markers for micronaire, 13 markers for strength, and 6 markers for elongation. Association between trait observations and marker regions were investigated by association analysis/mapping (-10LOG(p)" ≥ 3): 3 markers showed marker-trait association with fiber length, 1 marker with uniformity, 1 marker with micronaire, 15 markers with strength, and 10 markers with elongation.

In order to map resistant genes for Verticillium wilt, 'XinLuZao1', a susceptible cultivar *Gossypium hirsutum* L. and 'Hai7124', a resistant line *Gossypium barbadense* L., and their

$F_{2:3}$ families were studied using SSRs. Four and five QTLs were detected based on the disease index investigated on July 22 and August 24 in 2004 respectively and two marker intervals detected same QTLs.

An intraspecific $F_2$ segregation population of DH962 (an introgression line of *G. thurberi* with good fiber quality) and Jimian5 was also constructed. This map consisted of 139 loci mapped into 32 linkage groups, covered 1018.5cM and the mean interlocus distance was 9.08cM. QTL mapping for yield and fiber related traits revealed a total of 14 QTLs, six for yield-related traits and eight for fiber-related traits.

Now, marker assisted backcrossing have been conducted to apply these QTLs.

# Production and Characterization of Asymmetric Hybrids between Upland Cotton Coker201 (*Gossypium hirsutum*) and Wild Cotton (*Gossypium klozschianum* Anderss)

***Xiyan Yang, Xianlong Zhang*, Shuangxia Jin, Lili Fu, Linggang Wang***

National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, China

Asymmetric somatic hybrids were obtained between *Gossypium hirsutum* Coker 201 and wild cotton *G. klozschianum* Anderss. An investigation on the effect of UV irradiation on donor protoplast was carried out, and the lethal dose was determined to be 387 J/m$^2$. We firstly screened the putative hybrids by the color of the calli produced, followed by morphological, cytological and molecular analysis of putative hybrid plants. Most regenerated plants derived from fused protoplasts displayed a recipient-like morphology, while some showed an intermediate phenotype between Coker 201 and *G. klozschianum*. Chromosome numbers in these somatic hybrids ranged from 54 to 74. The hybrids were verified by random amplified polymorphism DNA (RAPD) and simple sequence repeat (SSR). Absence or co-existence of parents' genome DNA fragment was identified through molecular analysis. The heredity of cytoplasm was investigated by cleaved amplified polymorphic sequence (CAPS) analysis using mitochondrial and chloroplast universal primer pairs. The results indicated that recombination and rearrangements might have occurred in some regions of mitochondria (mt) and chloroplast (cp) DNA. Out of our knowledge, this is the first report about asymmetric cell fusion in cotton, and the hybrids obtained would be useful for breeding programs.

# Applying “Omics”-based Technology to Medicinal Plant Research

***David R. Gang***

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Herbs and spices have been used for thousands of years as foods and as medicines. The important medicinal properties of these plants are attributed to the compounds that they produce and accumulate. For example, sweet basil plants produce many compounds in their peltate glandular trichomes and ginger and turmeric produce similar compounds in their rhizomes that are important not only for plant defense, physiology, and interaction with the environment but that are also highly valued as flavoring/fragrance additives and as medicinal compounds. The curcuminoids and gingerols have been implicated as the major anti-inflammatory and anti-nausea compounds in turmeric and ginger. We are combining traditional biochemical and molecular biology approaches with a combination of functional genomics-, proteomics-, and metabolomics-based approaches to investigate not only the properties of these plants, but also the biosynthesis of important medicinal compounds. Using this approach we have identified a large number of compounds related to known medicinal compounds, and we have identified the genes encoding enzymes involved in production of many of these compounds.

# High-throughput Methods for Analyzing Gene Expression in Higher Plants

***David W. Galbraith***
Department of Plant Sciences and BIO5 Institute, University of Arizona, Tucson, AZ

Gene expression relates the information content of the genome to the individual phenotypes characterizing the different cell-types found within eukaryotic organisms. We are devising methods to provide a comprehensive description of gene expression within all cell types of model and crop plants, with the aim of employing these methods and the results that they provide, to obtain a detailed understanding of the mechanisms that regulate gene expression. These methods combine marker gene expression (particularly the Fluorescent Proteins), confocal and multiphoton microscopy, fluorescence-activated sorting, and expression, genotyping and protein microarrays. My talk will provide an overview of our progress in this area, as well as details of our recent discoveries.

# Gene Pyramiding to Improve Hybrid Rice by Molecular Marker-aided Selection

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Shanyou 63, a cross between Zhenshan 97 and Minghui 63, is an elite hybrid widely used in rice production in China. In recent years, however, this hybrid has shown several major problems in rice production including: the loss of resistance to diseases such as bacterial blight and fungi blast, in addition to its relatively poor cooking and eating quality. There is also urgent need for improving its resistance to insects such as stem borers and planthopper, as they frequently cause damage to the crop. We have conducted a series of work in order to solve the above problems: (1) *Xa21* and *Xa7*, two wide spectrum BB resistance gene, were introgressed to the restorer Minghui 63 by molecular marker aided selection to improve its resistance to bacterial blight. (2) *A Bt* δ-endotoxin gene was transformed to Minghui 63 to improve the stem borer resistance. (3) *Xa21* and *Bt* genes were combined into a line under the background of Minghui 63. (4) The allele at the *Wx* locus from Minghui 63 was transferred to Zhenshan 97 to improve the cooking and eating quality of this hybrid, resulting in a new version Zhenshan 97 with medium amylose content (AC), soft gel consistency (GC) and high gelatinization temperature (GT). (5) Two genes, *Pi1* and *Pi2*, showing broad-spectrum resistance to fungi blast, were introgressed into Zhenshan 97 to improve the blast resistance by molecular marker-aided selection. (6) Two genes, *Qbph1* and *Qbph2*, both highly resistant to brown planthopper, were introgressed to Zhenshan 97 and Minghui 63. The above versions of the lines can be combined in various ways to make new hybrids to meet the need of rice production.

**Key words:** Bacterial blight; Fungi blast; Bt; Brown planthopper; MAS; *Oryza sativa* L

# *Citrus* Genetic Improvement and Breeding in China

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*Citrus* is the most commercially important fruit crop and world-widely cultivated in sub-tropic areas. Germplasm preservation and method innovation in breeding are of great importance to the sustainability of *Citrus* industry. Citrus research group of the National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, is an important scientific research group in China. Researches in resent years of this group are mainly as follows.

*Somatic hybridzation aimed at rootstock and variety improvement or intermedial parents creation for ploidy breeding*

To date, more than 40 fusion combinations were conducted and the recovered progeny were transplanted, most of these plants began to flower and set fruit. Field performance evaluation proved that these plants possesses great potential as rootstock candidate, such as the hybrids of Hongju tangerine plus trifoliate orange, and intermedial parents for ploidy crossing, including those tetraploidy fused by two complementary edible parents; or directly fertilizing commercially important seedy cultivars, like Shatianyou pummelo, to produce seedless fruit.

*Improvement of local varieties*

Main objective of this project was seedlesslization of seedy local varieties, for example, ‘Ponkan’ tangerine and common sweet oranges have being genetically improved by triploidylization or immigrating CMS traits by biotechnology; adjustment of the harvest time for very early or late maturity; and quality breeding to enhance fruit functional components or surface color are also performing.

*Mutant investigation and evaluation*

This research also conducted for the purpose of polyploidy isolation or morphology diversity using for novel variety breeding by widely screening the field-mutant across the main citrus produce areas in China. Mechanisms of bud-sport in *Citrus* are undergoing in recent years through a set of analyses methods such as cDNA-AFLP and microarray, and physiological assays are also conducting by HPLC and CE.

*Germplasms collection, evaluation and application*

Wild and ancient local *Citrus* germplasms in south China were investigated and more then 30 accessions were grafted in the Center of Citrus Breeding of HZAU. Calluses from most of these germplasms such as Mongshan, Daoxian and Congyi wild mandarins were induced, and have been adopted as explants for protoplast fusion to transfer the desirable resistant traits to the main cultivars.

# Research Progress of Mechanism Underlying Lycopene Accumulation in *Citrus* Juice Sacs

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Pink or red juice sacs of *Citrus* (here only refers to lycopene-pigmented ones) are competitive in market because the pigment of carotenoids represents not only the pleasant color but also a group of healthy anti-oxidants, which play important roles in the prevention of cancer and chronic diseases. Whether *Citrus* could accumulate more specific carotenoids to meet various desires, or the composition of *Citrus* carotenoids could be artificially controlled is among interesting questions as well as pursuits arise from the lycopene-pigmented *Citrus*. So far, the mechanism underlying the carotenoids accumulation still remains unknown, which is the key point to make clear before proposing above questions or pursuits.

In our efforts to elucidate the mechanism, biophysiological and molecular characteristics were studied on pink-juice-sacs sweet oranges (*Citrus sinensis* Osbeck) of. Cara Cara navel orange and Honganliu sweet orange, both oranges, are originated as spontaneous bud mutation from pale-yellow fleshed Washington navel orange and Anliu sweet orange respectively.

Physiological data showed that the colorless and also the precursor of carotenoids phytoene take the highest carotenoids in Cara Cara navel oranges with the contents of 173 μg/g (DW), and followed by lycopene with 69 μg/g (DW). In contrast, lycopene takes the highest carotenoids as 36 μg/g (FW) in honganliu sweet orange, while phytoene takes the second place as 12 μg/g (FW). These results implied that different mutation mechanism might present in two oranges. Furthermore, both α- and β-carotene are also accumulated in a less extent inside the juice sacs of both pink-flesh sweet oranges compared to their original cultivars.

From gene cloning and expression analysis on Cara Cara navel orange, we knew that there are 2 copies of phytoene synthase gene, the expression of the fruit-specific one is abnormal compared with the control; Both lycopene β- and ε-cyclases genes were found more than one transcribes; none of the 6 genes encoding geranylgeranylpyrophosphate synthase, phytoene desaturase，ζ- carotene desaturase，*β*-carotenoid hydroxylase, zeaxanthin epoxidase and carotenoid isomerase is account for the pigment accumulation.

Expression analyses on Honganliu sweet orange showed that the expression of

upstream genes encoding phytoene synthase, phytoene desaturase and ζ- carotene desaturase were up regulated in juice sacs. The gene expression levels were found corresponded with lycopene content in above tissues.

Thus, though different mechanisms may underlie the two lycopene-pigmented oranges, but the regulator(s) that affect expression of upstream genes esp. phytoene synthase maybe the common key factor that attracts our interesting in further researches.

# Protoplast Technology and *Citrus* Genetic Improvement

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*Citrus* is one most important fruit crop in China and worldwide. Protoplast fusion has been an effective and successful technique for citrus improvement by circumventing reproductive barriers such as nucellar polyembryony, pollen/ovule sterility, sexual incompatibility and long juvenility encountered in conventional breeding. Protoplasts isolated from embryogenic callus line, as source material are convenient and available at any time for genetic transformation of seedless cultivars since most commercial cultivars are seedless, and routinely used epicotyl seedling segments are only available for seedy cultivars. In our program, somatic hybrids from more than 30 interspecific and intergeneric fusion combinations have been produced. By symmetric fusion between embryogenic callus protoplasts of Satsuma mandarin (CMS type with sterile cytoplasm) and mesophyll protoplasts of elite seedy cultivars, diploid cybrid plants containing sterile cytoplasm from Satsuma were regenerated from seven combinations. Transgenic plants were produced following protoplast transformation with the gfp (green fluorescent protein) gene mediated by polyethylene glycol. Since *citrus* mesophyll protoplasts usually do not divide and regenerate, by fusion of embryogenic callus protoplasts with gfp transgenic mesophyll protoplasts, facilitated by gfp expression and visualization in hybrid cells, somatic hybrid vigor or regeneration advantage was revealed and evidenced. Currently, using the fusion model of gfp transgenic embryogenic callus protoplasts of CMS type Satsuma mandarin with mesophyll protoplasts from seedy cultivars, studies on mechanisms for cybrid regeneration as well as efficient GFP negative selection of cybrid types via symmetric fusion are being conducted; meanwhile, these regenerated somatic hybrids are being evaluated for *citrus* scion and rootstock improvement.

# *Citrus* Genetic Transformation with Interest Target Genes for Cultivar Improvement

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*Citrus* is one most important fruit crop, genetic transformation with more recently cloned target genes is an alternative effective way for citrus improvement. In our program, transformation systems using several explant sources such as protoplasts, embryogenic calluses and seedling epicotyledon segments have been well established and optimised. Protoplast and embryogenic callus as explants are convenient, and available at any time for transformation of seedless cultivars. Epicotyledon segments from seed germination as explants are only available for seedy cultivars. The organogenesis of epicotyl segments of 'Bingtang' sweet orange (*Citrus sinensis* L. Osbeck), an elite citrus cultivar in China was optimized recently. Different cut modes (i.e. transversal cut, longitudinal cut and oblique cut) were compared and their effect on the number and quality of regenerated buds and shoots was observed. Oblique cut, which was first reported, showed the best result. With the optimized regeneration systems, genetic transformation with several target genes such as *green fluorescent protein* (*GFP*, an *in vivo* visual marker), *LFY*, *AP1* (to shorten juvenility), *Xa21* (for potential citrus bacterial canker resistance), and *Barnase* (to induce seedless fruit) was conducted and numerous transgenic plants were regenerated from several citrus cultivars. Southern blot analysis confirmed the integration of target genes in the plant genome. These transgenic materials are valuable for both basic research and for further evaluation in cultivar improvement.

# From Genetic Bases to Molecular Bases for Rice Spikelets per Panicle

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The number of spikelets per panicle (SPP) is a typical quantitative trait, which makes an important contribution to rice grain yield. Eight mapping populations (DH, RIL) derived from different parents with varied SPP have been used for dissection of its genetic bases since 1997. Totally, over 20 QTL for SPP were detected on 12 chromosomes among these populations. Epistasis was also shown to be an important factor affecting the trait. In the past five years, more attention was paid to develop near isogenic lines (NILs) for main effect QTLs concerning their easy manipulation. Currently, 14 NILs for QTLs, including 8 major QTLs and 6 minor QTLs commonly detected at least in two populations, were developed by consecutive backcrosses. Of them, *qSPP7-1*, *qSPP7-2* and *qSPP8* all were fine mapped to a small region of less than 20 kb. 4 QTLs (*qSPP1-c*, *qSPP2*, *qSPP4* and *qSPP10*) are in the process of fine mapping using large NIL-F2 populations. So far, *qSPP7-1* candidate gene was identified and used for transformation, the transgenic plant showed the expected trait changes, highly in agreement with the phenotype of NIL- *qSPP7-1*. Attempts to discover its molecular mechanism is being made by several biological methods including yeast two-hybrid system, electrophoretic mobility shift assay (EMAS), Chromatin immunoprecipitation (ChIP) and gene array. From now on, our research activity for SPP is gradually moving towards the molecular bases from genetic bases via cloning individual QTL.

# Generation of Introgression Lines of Wild Rice for Gene Discovery

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The genus *Oryza* contains two cultivated species, the Asia cultivar *O. sativa* and Africa cultivar *O. glaberrima*, and 22 wild species. The *O. sativa* has two subspecies. The wild species of genus *Oryza* display enormous differences from the cultivated ones in developmental, morphological and adaptive traits, providing invaluable resources that can be used to increase the genetic diversity of cultivated rice. Therefore, the genome-wide comparative analyses among species, subspecies and cultivars in the genus *Oryza* will have great impacts on rice functional genomics and breeding. In order to understand the effect of gene flow between wild rice and *O. sativa*, and to analyze functional genes conferring physiological, developmental and agronomical traits with potential for rice improvement, we have developed a series of introgression lines (ILs) involved the subspecies (*Indica* and *Japonica*). Two ILs populations with overlapping introgression segments from the sequenced *japonica* cv. Nipponbare into the uniform genetic background of two elite *indica* hybrid parental lines Zhenshan 97B and 93-11 have been obtained recently. The ILs was generated by repeated backcrosses with marker-aided selection. Above one hundred seventeen polymorphic simple sequence repeats markers evenly distributed on rice genome were used to monitor the *japonica* fragment introgression through $BC_3$ or $BC_4$ to the end of target ILs reached that each contained a single chromosomal segment. Two sets of homozygous ILs were finally selected as to that all lines with overlapping introgression chromosomal regions of the *japonica* together covers entire rice genome. Because the ILs could be treated as paired near-isogenic resource, which does largely minimize the phenotyping deviations from the heterogeneity of growth stage within experiments and the masking effects of epistatic interactions, they represent a unique system for gene mapping purposes. Using a set of 135 homozygous ILs with Zhenshan 97B background, we have conducted quantitative trait loci (QTL) analysis for important traits such as plant height, heading date and yield components. The discovery of larger numbers of QTL were identified and several may be newly found for the traits，reflects the ability of the ILs to resolve smaller QTL effects. The results provide useful information that helps understand genetic basis of the trait changes in the introgression lines. We also initiated to generate a series of ILs from AA-genome wild accessions that used in OMAP into the elite hybrid parental lines through the similar backcross strategy. The genome-wide coverage ILs

with the wild segments in the elite cultivated background will be a permanent resource for QTL identifying, fine mapping and cloning of genes controlling the differences between the wild rice and the *O. sativa*.

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