56th Annual Maize Genetics Conference

Program and Abstracts

March 13 – March 16, 2014

Beijing, China

This conference received financial support from:

National Science Foundation
Monsanto
DuPont Pioneer
Dow AgroSciences
Syngenta
BASF Plant Science
KWS
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We thank these contributors for their generosity!

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Cover art by

Haijun Liu
National Key Laboratory of Crop Genetic Improvement
Huazhong Agricultural University

General Information

Meeting Registration

Thursday: 3:00 PM to 6:00 PM: There will be a table in the lobby of the hotel (see the plan of hotel level 1).
7:00 PM to 9:00PM: There will be a table in the lobby of the hotel (see the plan of hotel level 1).
Friday: 7:00AM to 8:15AM: There will be a table in the lobby of the hotel (see the plan of hotel level 1).

Meals

All meals will be served in the hotel, in the Grand Ballroom and the Li Jiang multifunctional room; serving hours as listed in the Program. Coffee, tea and soft drinks are available at no charge during the beverage breaks.

Talks and Posters

All Talks will be presented in Convention Hall Number 2, on the second floor.

Posters will be presented in the Exhibition Hall on the first floor. Posters should be hung Thursday starting at 3 PM and stay up until Sunday morning, but must be removed by 9 AM on Sunday. During poster sessions, presenters of odd number posters are asked to stand by their posters 1:30-3 PM on Friday and 3-4:30 PM on Saturday. Presenters of even numbered posters should stand by their posters 3-4:30 PM on Friday and 1:30-3 PM on Saturday.

The maize meeting is a forum for presentation and discussion of unpublished material. Photographing or recording of talks and posters is not allowed. For authors who give permission to view electronic copies of oral and/or poster presentations, pdf files will be available at MaizeGDB shortly after the meeting at the following URL:

http://maizegdb.org/maize_meeting/2014/downloads.php

Hospitality

After the evening sessions there will be informal socializing and poster gazing in the Exhibition Hall, with refreshments provided until 12:00 AM.

After 12:00 AM, the Cafe Restaurant is available for continued socializing (see the plan of hotel level 1).

Steering Committee

Please share your suggestions and comments about the meeting with the 2014 Steering Committee

Ann Stapleton, Chair..... (stapletona@uncw.edu)
Mark Settles, co-Chair ..... (amsettles@ifas.ufl.edu)
Jinsheng Lai, Local Host... (jlai@cau.edu.cn)
Peter Balint-Kurti..... (peter_balintkurti@ncsu.edu)
Alice Barkan ..... (abarkan@uoregon.edu)
Phil Becraft Phil Becraft ... (becraft@iastate.edu)
Wes Bruce..... (wes.bruce@basf.com)
Sherry Flint-Garcia ..... (sherry.flint-garcia@ars.usda.gov)
Milena Ouzunova..... (m.ouzunova@kws.com)
Ruairidh Sawers..... (rsawers@langebio.cinvestav.mx)
Amanda Wright..... (amanda.wright@unt.edu)

Ex officio:
Carson Andorf, abstract coordinator
Paula McSteen, Treasurer
Marty Sachs
Mary Schaeffer, abstract coordinator

Acknowledgements

Many thanks go to Carson Andorf, Darwin Campbell, and Mary Schaeffer for their tremendous efforts in organizing, assembling, and advertising the conference program. We also thank Angela Freemyer and her team at the University of Missouri Conference Center for helping to organize the conference, handling registration and dealing with a multitude of other issues. Special thanks are also extended to Weibin Song and the CAU staff for their help in organizing this conference.

Hotel Information:

Beijing North Star Continental Grand Hotel
Add:No.8 Beichen Dong Road, Chaoyang District, Beijing P. R. China 100101,
Room Reservations:(8610)84980105
(8610)84985588--Room Reservation Dept

E-mail:bcgh@bcghotel.com
Website:www.bcghotel.com

北辰五洲大酒店

地址：中国北京市朝阳区北辰东路 8 号 邮编：100101

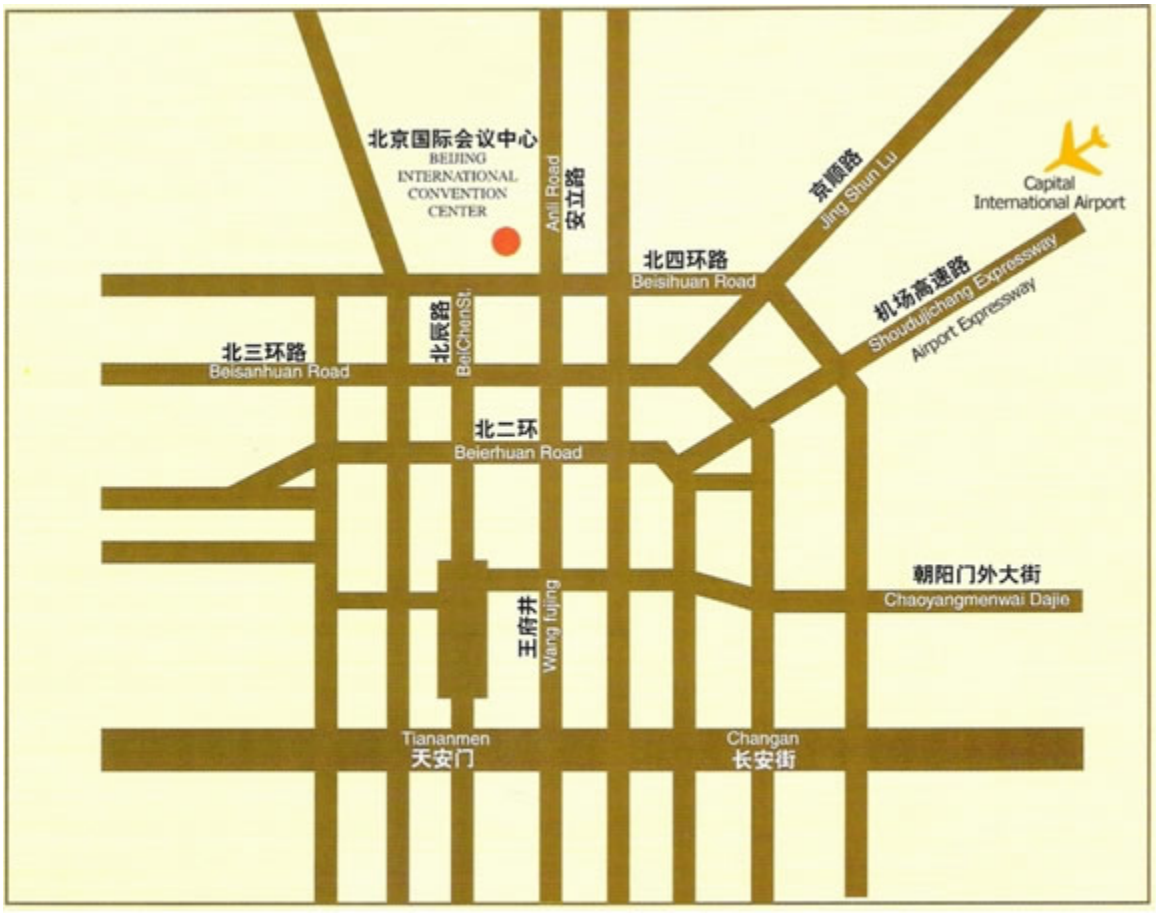
散客订房：(8610)84980105

(8610)84985588　转　客房预订部

电子邮件：bcgh@bcghotel.com

网　　址：www.bcghotel.com

Hotel Maps:

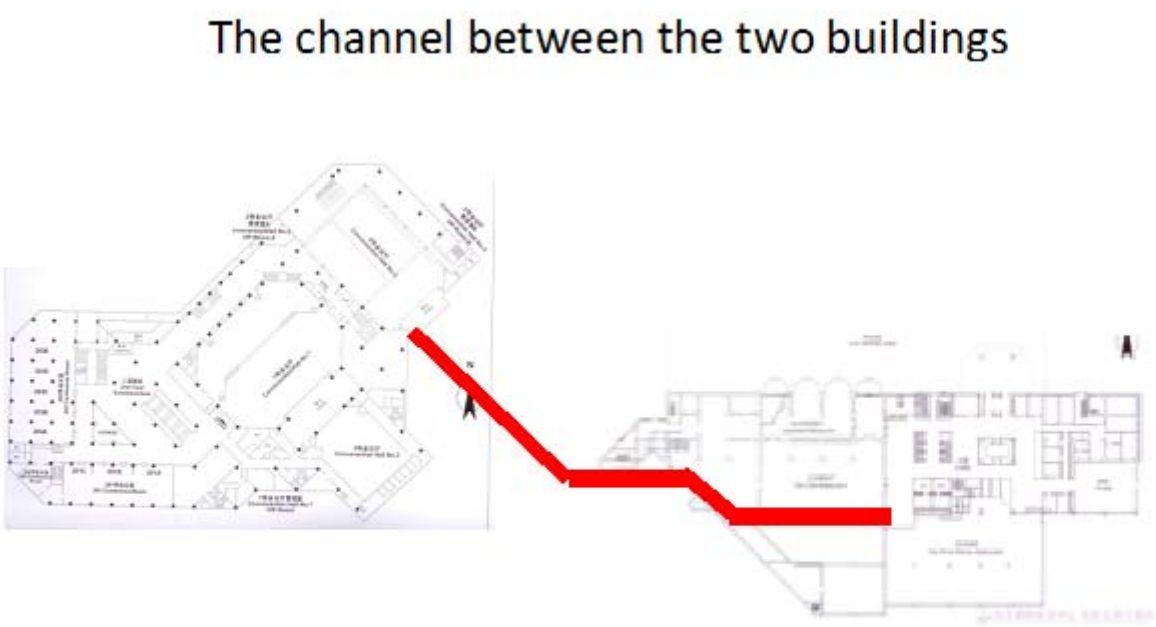

北京国际会议中心
BEIJING
INTERNATIONAL
CONVENTION
CENTER
Anli Road
安立路
京顺路
Jing Shun Lu
Capital
International Airport
北四环路
Beisihuan Road
机场高速路
Shoudujichang Expressway
Airport Expressway
北三环路
Beisanhuan Road
北辰路
BeiChenSt.
北二环
Beierhuan Road
朝阳门外大街
Chaoyangmenwai Dajie
王府井
Wang fujing
Tiananmen
天安门
Changan
长安街


Airport
北京国际会议中心
Beijing International
Convention Center

Map of convention center and hotel:

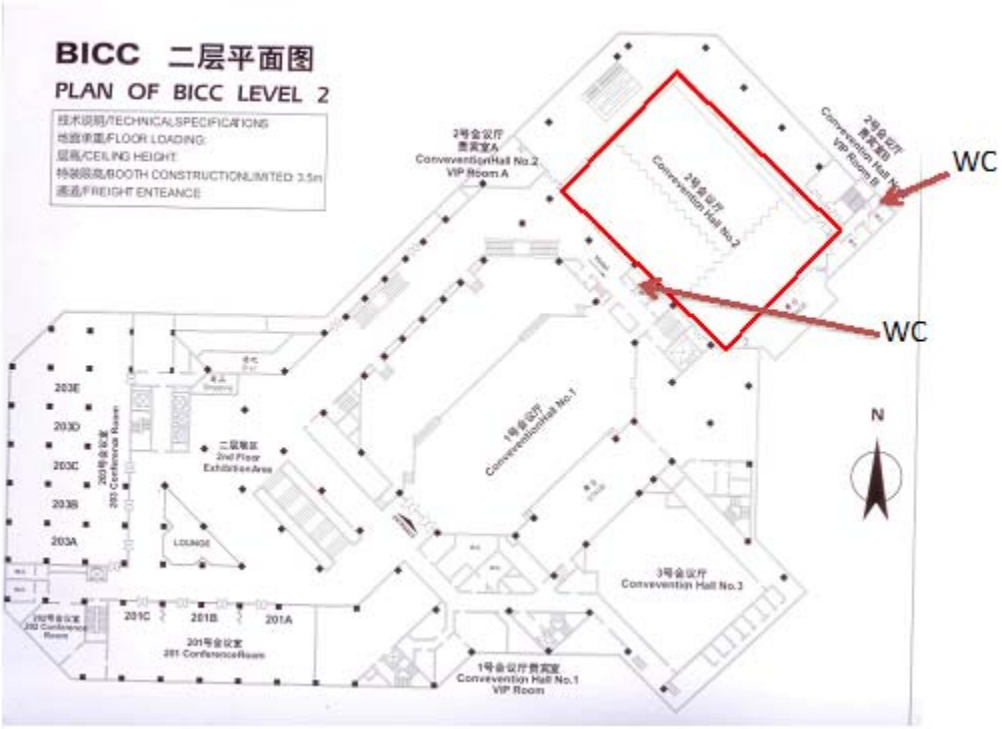
The two buildings:

The route between buildings:



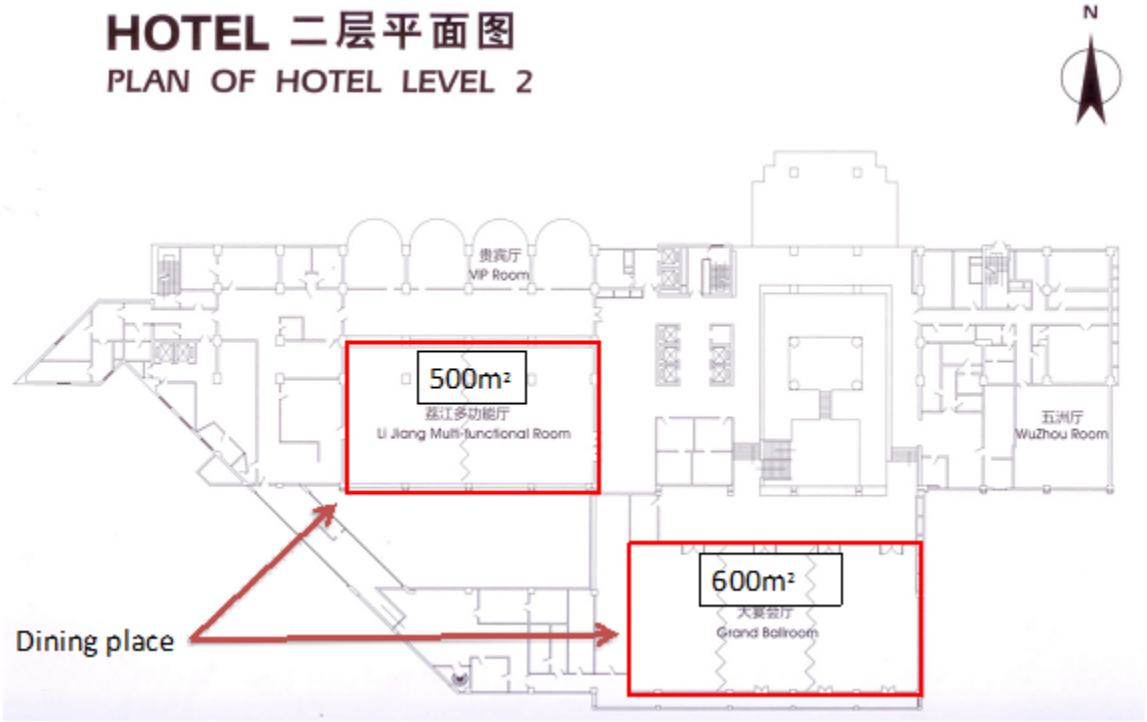
The width of the channel： 2.5 meter
The longth of the channel： 80 meter
Walking time on foot from the two buildings: 3minutes

Convention center map (Main venue: 2nd floor):

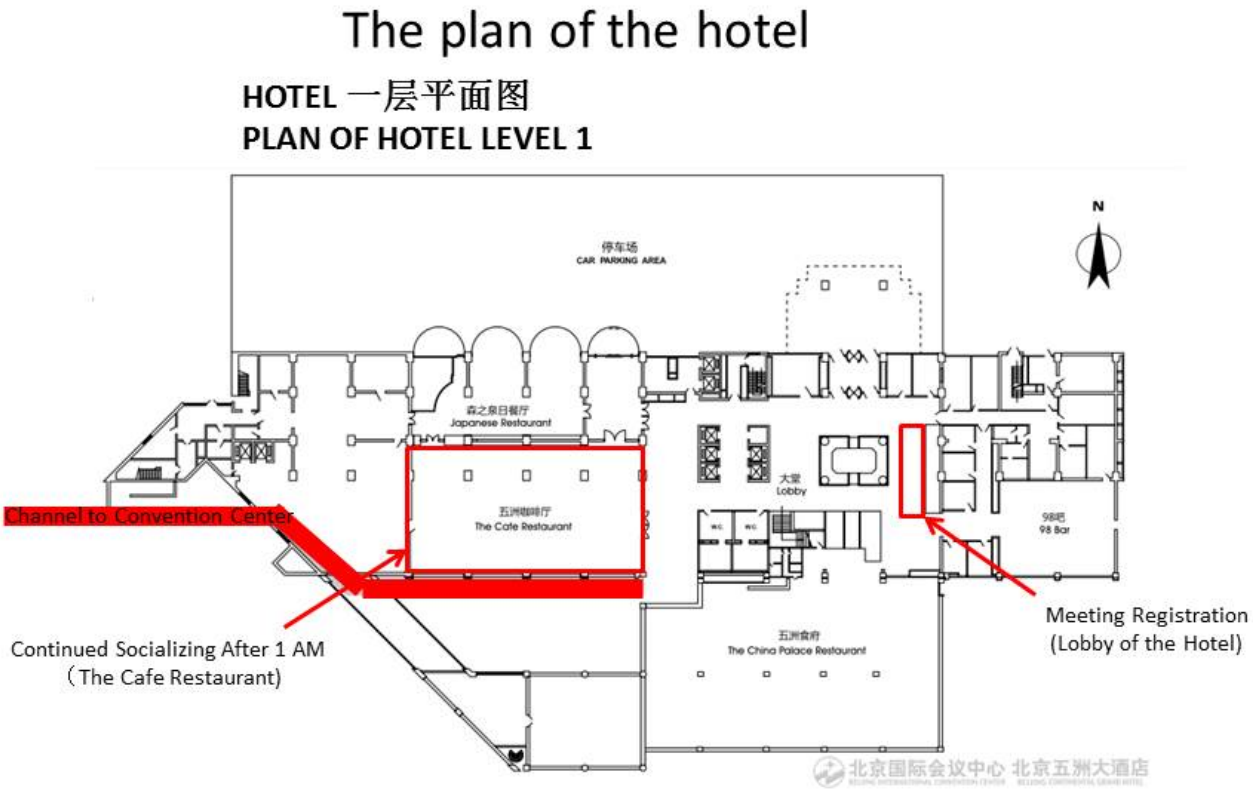


Exhibition Hall (Main venue: 1st floor):

Hotel dining area map:



Registration and socializing area:



Useful Links:

2014 Maize Meeting Website

http://maizegdb.org/maize_meeting/2014

2015 Maize Meeting Website (Available November 2014)

http://maizegdb.org/maize_meeting/2015

Abstract Book (Electronic version)

http://maizegdb.org/maize_meeting/abstracts/2014Program.pdf

Cover Image (High-quality color)

http://maizegdb.org/ maize_meeting/coverart/

Upload poster or talk

http://maizegdb.org/ maize_meeting/2014/downloads.php

Schedule of Events

Talks will be held in Convention Hall 2 on the second floor.
Posters will be displayed in the Exhibition Hall on the first floor.

Thursday, March 13

2:00 PM – 6:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**
12:00 PM – 2:00 PM	**Positional Cloning in Maize Workshop** (Conference Room 303)
2:00 PM – 3:00 PM	**MaizeGDB Basics Workshop** (Conference Room 303)
3:00 PM – 4:00 PM	**MaizeGDB Advanced Workshop** (Conference Room 303)
4:00 PM – 6:00 PM	**Gramene Workshop** (Conference Room 303)
	Pre-registration recommended for the above sessions.
3:00 PM – 6:00 PM	**REGISTRATION** (Hotel Lobby)
3:00 PM – 6:00 PM	**POSTER HANGING** (Exhibition Hall)
6:00 PM – 7:00 PM	**DINNER** (Hotel Dining Rooms)
7:00 PM – 9:00 PM	**SESSION 1 – PLENARY TALKS** Chair: Wes Bruce Pages 19 & 20
7:00 PM	**WELCOME AND ANNOUNCEMENTS** (Convention Hall #2) Ann Stapleton and Wes Bruce
7:15 PM	**Tom Brutnell, Donald Danforth Plant Science Center** [Plen 1] *A systems approach to understanding photosynthetic differentiation in the grasses*
8:05 PM	**Jiayang Li (李家洋), Chinese Academy of Sciences & Chinese Academy of Agricultural Sciences** [Plen 2] *Molecular basis for designing high-yield and good-quality rice*
7:00 PM – 9:00 PM	**REGISTRATION** (Hotel Lobby)
9:00 PM – 11:59 PM	**INFORMAL POSTER VIEWING & HOSPITALITY** (Exhibition Hall)
12:00 AM	**HOSPITALITY** (Café Restaurant)

Friday, March 14

7:00 AM – 8:00 AM	**BREAKFAST** (Hotel Dining Rooms)
7:00 AM – 8:15 AM	**REGISTRATION** (Hotel Lobby)
8:15 AM – 9:55 AM	**SESSION 2 - DEVELOPMENTAL GENETICS, QUANTITATIVE GENETICS I** Chair: Peter Balint-Kurti Talks 1-5. Pages 24 - 28
8:00 AM	**ANNOUNCEMENTS** (Convention Hall #2) Ann Stapleton and Jinsheng Lai
8:15 AM	**George Chuck, USDA-ARS** [T1] *The SPB-box transcription factors unbranched2 and unbranched3 function redundantly with tasselsheath4 to regulate plastochron index*
8:35 AM	**Davide Sosso, Stanford University** [T2] *SWEET transporters and seed filling: tools for plants or opportunities for pathogens?*
8:55 AM	**Andrea Gallavotti, Rutgers University** [T3] *Auxin signaling in the early steps of maize inflorescence development*
9:15 AM	**Madelaine Bartlett, Brigham Young University** [T4] *The B-class mutant sterile tassel silky ear1 (sts1) provides a window into both conserved and divergent aspects of maize floral development*
9:35 AM	**Rachel Egger, Stanford University** [T5] *Transcriptomes and Proteomes Define Gene Expression Progression in Pre-meiotic Maize Anthers*
9:55 AM	**BREAK**
10:30 AM – 12:10 PM	**SESSION 3 – DEVELOPMENTAL GENETICS, QUANTITATIVE GENETICS II** Chair: Feng Tian Talks 6-10. Pages 29 - 33
10:30 AM	**Bao-Cai Tan, The Chinese University of Hong Kong** [T6] *Small kernel1 encodes a pentatricopeptide repeat protein required for mitochondrial nad7 transcript editing and seed development in maize and rice*
10:50 AM	**Andrea Eveland, Cold Spring Harbor Laboratory** [T7] *Regulatory networks controlling maize inflorescence architecture and the interface with early season drought responses*
11:10 AM	**Chung-Ju Rachel Wang, Academia Sinica** [T8] *DSY2 is required for meiotic recombination and synaptonemal complex formation*
11:30 AM	**Peter Bradbury, USDA-ARS** [T9] *The distribution of recombination breakpoints and their association with genomic features in two maize NAM populations*
11:50 AM	**Guohua Mi, China Agricultural University** [T10] *Enhancement of phosphorus efficiency through genetic improvement of root architecture in maize*

Friday, March 14 (continued)

12:30 PM – 1:30 PM **LUNCH** (Hotel Dining Rooms)

1:30 PM – 5:00 PM **POSTER SESSION 1** (Exhibition Hall)

1:30 PM – 3:00 PM *Presenters should be at **odd** numbered posters.*
3:00 PM – 4:30 PM *Presenters should be at **even** numbered posters.*

Beverages will be available from 3:30 PM to 5:00 PM.

4:00 PM – 6:00 PM **OPTIONAL CONFERENCE WORKSHOP**

4:00 PM – 6:00 PM **iPlant Collaborative Workshop** (Conference Room 303)

Pre-registration recommended for the above workshop.

6:00 PM – 7:00 PM **DINNER** (Hotel Dining Rooms)

7:00 PM – 8:15 PM **SESSION 4 – MCCLINTOCK PRIZE WINNER**
Chair: Jeff Bennetzen McClintock talk 1. Page 23

7:00 PM **Introduction**
Jeff Bennetzen

7:10 PM **Prof. Sir David Baulcombe, University of Cambridge** [M1]
When genomes meet – RNA, epigenetics and phenotypes of hybrid plant

8:15 PM – 11:59PM **INFORMAL POSTER VIEWING & HOSPITALITY**
(Exhibition Hall)

12:00 AM **HOSPITALITY**
(Café Restaurant)

Saturday, March 15

7:00 AM – 8:00 AM **BREAKFAST** (Hotel Dining Rooms)

8:00 AM – 9:55 AM **SESSION 5 – BIOCHEMICAL GENETICS, QUANTITATIVE GENETICS, RESOURCES I**
Chair: Gernot Presting Talks 11-15. Pages 34 - 38

8:00 AM **ANNOUNCEMENTS** (Convention Hall #2)
Ann Stapleton and Jinsheng Lai

8:15 AM **Bailin Li, DuPont Pioneer** [T11]
Cloning and Characterization of a Major QTL for Northern Leaf Blight Resistance from Two Resistant Lines in Maize

8:35 AM **Mingliang Xu, China Agricultural University** [T12]
A wall-associated kinase ZmWAK confers quantitative resistance to head smut in maize

8:55 AM **Xiquan Gao, Nanjing Agricultural University** [T13]
Functional genomics analysis of maize 9-lipoxygenase gene family and 9-oxylipins in the basal resistance to ear rot and stalk rot caused by Fusarium spp. and the induced systemic resistance to anthracnose leaf blight by Colletotrichum graminocola

9:15 AM **Alex Lipka, Cornell University** [T14]
Inference of metabolic pathway dynamics through quantitative genetics: a story of maize grain carotenoids in the nested association mapping panel

9:35 AM **Jinliang Yang, Iowa State University** [T15]
GWAS for Trait-Associated SNPs that Exhibit Dominance Effects Provides Insight Into the Origin of Heterotic Groups

9:55 AM **BREAK**

10:30 AM – 12:10 PM **SESSION 6 – BIOCHEMICAL GENETICS, QUANTITATIVE GENETICS, RESOURCES II**
Chair: Milena Ouzenva Talks 16-20. Pages 39 - 43

10:30 AM **Mei Guo, DuPont Pioneer** [T16]
Maize ARGOS1 (ZAR1) Transgenic Alleles Increase Hybrid Maize Yield

10:50 AM **Xiaohuan Sun, VIB and Ghent University** [T17]
Mild drought specifically affects the transition between cell division and cell expansion in the growing maize leaf

11:10 AM **Leandro G. Neves, RAPiD Genomics** [T18]
Maize Genotyping using RAPiD-Seq (Randomly-Amplified Polymorphic DNA Sequencing)

11:30 AM **Doreen Ware, USDA-ARS; Cold Spring Harbor Laboratory** [T19]
Gramene: A Resource for Comparative Plant Genomics

11:50 AM **Gerry Neuffer, University of Missouri** [T20]
Mutagenesis; characterization and evaluation

Saturday, March 15

12:30 PM – 1:30 PM	**LUNCH** (Hotel Dining Rooms)
1:30 PM – 5:00 PM	**POSTER SESSION 2** (Exhibition Hall)
1:30 PM – 3:00 PM	*Presenters should be at **even** numbered posters.*
3:00 PM – 4:30 PM	*Presenters should be at **odd** numbered posters.*

Beverages will be available from 3:30 PM to 5:00 PM.

4:30 PM – 5:45 PM	**COMMUNITY SESSION - Maize Genetics Executive Committee** MGEC Chair: Jeff Bennetzen
6:00 PM – 7:00 PM	**DINNER** (Hotel Dining Rooms)
7:15 PM – 8:55 PM	**SESSION 7 – PLENARY TALKS** Chair: David Jackson Pages 21& 22
7:15 PM	**Graeme Hammer, University of Queensland** [Plen 3] *Molecular breeding for complex adaptive traits – how integrating crop ecophysiology and modelling can enhance efficiency*
8:05 PM	**Toby Kellogg, Donald Danforth Plant Science Center** [Plen 4] *Polyploidy in maize and its relatives*
9:00 PM – 11:59 PM	**INFORMAL POSTER VIEWING** (Exhibition Hall)
12:00 AM	**HOSPITALITY** (Café Restaurant)

Sunday, March 16

7:00 AM – 8:20 AM **BREAKFAST** (Hotel Dining Rooms)

Posters should be taken down by 9 am!

8:20 AM – 10:10 AM	**SESSION 8 – DEVELOPMENTAL GENETICS, GENOME I** Chair: Phil Becraft	Talks 21-25. Pages 44 - 48
8:20 AM	**ANNOUNCEMENTS** Ann Stapleton and Jinsheng Lai	(Convention Hall #2)
8:30 AM	**Faqiang Li, University of Wisconsin** *Characterization of Maize Autophagy System Reveals a Central Role in Nitrogen Remobilization*	[T21]
8:50 AM	**Phil Taylor, Monsanto Company** *Expression of Arabidopsis thaliana HB17 gene in corn leads to improved sink potential*	[T22]
9:10 AM	**Cristian Forestan, University of Padova** *ZmHDA108 has an active role both in setting the histone code and controlling plant vegetative and reproductive development of maize.*	[T23]
9:30 AM	**Bob Meeley, DuPont Pioneer** *Impairment of a Chitinase-like1 is responsible for the phenotype of a brittle stalk mutant of maize*	[T24]
9:50 AM	**Felix Seifert, University of Hamburg** *Distinct small RNA populations act antagonistically in heterosis formation of maize*	[T25]
10:10 AM	**BREAK**	
10:30 AM – 11:50 AM	**SESSION 9 – DEVELOPMENTAL GENETICS, GENOME II** Chair: Mark Settles	Talks 26-29. Pages 49 - 52
10:30 AM	**Silong Sun, China Agricultural University** *De novo assembly of Maize Mo17 genome and its comparison with B73 genome*	[T26]
10:50 AM	**Haijun Liu, Huazhong Agricultural University** *Genomic, transcriptomic and phenomic variation reveals the complex adaptation of modern maize breeding*	[T27]
11:10 AM	**Jeff Gustin, University of Florida** *Machine vision phenotyping uncovers novel relationships between kernel and seedling characteristics*	[T28]
11:30 AM	**Jeff Bennetzen, University of Georgia** *Gene and genome changes in the grasses*	[T29]
11:50 AM	**ADJOURNMENT**	

Posters

Computational and Large-Scale Biology

P1	**Zhiwu Zhang** <zz19@cornell.edu>	A Lightened Path for Dissecting Maize Flowering Time by Using Genome-Wide Association Study
P2	**Zexi Cai** <caizexi123@cau.edu.cn>	Analysis of the repeat sequences in *Coix lachryma-jobi* and *Coix aquatic* genomes
P3	**Edward Buckler** <esb33@cornell.edu>	Biology of Rare Alleles in Maize and Its Wild Relatives
P4	**Raffaella Battaglia** <raffaella.battaglia@unicatt.it>	Breeding maize for resistance to Fusarium ear rot: a candidate gene approach from the integration of metabolomics and transcriptomics
P5	**Dezhou Cui** <dezhoucui@126.com>	Comparative Proteomic Analysis of Salt Stress Responses in the Roots of Two Maize (*Zea Mays* L.) Inbred Lines at the Early Seedling Stage
P6	**Huanhuan Tai** <hhtai@uni-bonn.de>	Comparative transcriptomic analysis of primary, seminal and crown roots of maize
P7	**Paul Bilinski** <pbilinsk@gmail.com>	Comparing Rates of Changing Repetitive Content to Rates of Genic Nucleotide Polymorphism in Maize Landraces and Their Relatives in *Zea* and *Tripsacum*
P8	**Tifu Zhang** <zhangtifu_82@163.com>	Deep analysis on maize genome sequencing data reveals that the presence/absence variation (PAV) was comprehensively involved in disease resistance
P9	**Qiuyue Chen** <qych@cau.edu.cn>	Dissect the Genetic Architecture of Nitrogen Response in Maize
P10	**Matthew Hufford** <mhufford@iastate.edu>	Evaluating Evidence for Centromere Drive in the Family Poaceae
P11	**Lifang Zhang** <zhangl@cshl.edu>	Exploring Gene Regulatory Network of miRNAs in Maize
P12	**Tom Hughes** <thomas.hughes@plants.ox.ac.uk>	Extensive regulatory neofunctionalisation following whole genome duplication in maize
P13	**Candice Hirsch** <cnhirsch@umn.edu>	From seed to senescence: Transcriptome tools to understand maize development, physiology, and phenotypic diversity
P14	**Kokulapalan Wimalanathan** <kokul@iastate.edu>	Functional annotation of B73 gene models: A machine learning approach
P15	**Carson Andorf** <carson.andorf@ars.usda.gov>	G-quadruplex motifs are found in genes regulated by hypoxia, low sugar, and nutrient deprivation in maize
P16	**Taner Sen** <taner.sen@ars.usda.gov>	Metabolic Pathway Tools and Resources at MaizeGDB
P17	**Zhengbin Liu** <liuzhen@missouri.edu>	Genome-wide Appraisal of Loss-of–Function Variants in Zea mays
P18	**Yan He** <heyan1111@hotmail.com>	Genomic features shaping the landscape of meiotic double strand break hotspots in maize

Computational and Large-Scale Biology (Continued)

P19	**Fei Lu** <fl262@cornell.edu>	Genotyping by sequencing on a worldwide collection of maize inbreds, landraces and teosintes
P20	**Wei Zhang** <wzhang@waksman.rutgers.edu>	Histone modification profiles are different between leaf and endosperm in maize
P21	**Wenwei Xiong** <xiongwenwei@gmail.com>	Integrated network analysis and inference on maize endosperm development
P22	**Caroline Marcon** <marcon@uni-bonn.de>	Large-scale proteomic and phospho-proteomic analyses of maize root tissues
P23	**Yuanda Lv** <lyd0527@126.com>	Long non-coding RNAs responsive to nitrogen deficiency in maize leaves
P24	**Yinping Jiao** <yjiao@cshl.edu>	Maize Pan-genome construction by short reads assembly
P25	**Hanwei Yan** <qiji19870814@163.com>	PIGD: A database for intronless genes in the Poaceae
P26	**Guoying Wang** <wangguoying@caas.cn>	RNA sequencing reveals the complex regulatory network in the maize kernel
P27	**Robert Schaefer** <schae234@umn.edu>	Systems biology approaches for integrating datasets identifying genes related to iron nutritional quality in maize
P28	**Jason Williams** <williams@cshl.edu>	The *iPlant Collaborative*: A Unified Cyberinfrastructure for Plant Science
P29	**Christy Gault** <cgault@ufl.edu>	The N-terminus of the ROUGH ENDOSPERM3 splicing factor is necessary for splicing U12-type introns
P30	**Yanxin Zhao** <rentlang@webmail.hzau.edu.cn>	The structure and evolution of *mTERF* gene families
P31	**Shan Wu** <wus@ufl.edu>	The UniformMu transposon resource for functional genomics
P32	**Wilson Huanca-Mamani** <whuanca@uta.cl>	Transcriptional profiling of lluteño maize under salt stress and excess of boron, a northern Chile maize highly tolerant to the abiotic stress
P33	**Wenbin Mei** <wmei@ufl.edu>	When Alternative Splicing Meets Whole Genome Duplication and Gene Body Methylation

Biochemical and Molecular Genetics

P34	**Qi-Jun Chen** <qjchen@cau.edu.cn>	A CRISPR/Cas-based Toolkit for Maize Multiplex Genome Editing, Multigene Interference, and Multigene Activation
P35	**Paul Scott** <paul.scott@ars.usda.gov>	A semi-in vitro system for studying genetic compatibility
P36	**Joanna Gracz** <j.gracz@gmail.com>	Alternative splicing events in two maize lines under herbicide stress conditions
P37	**Fei Gao** <gaofeii@263.net>	An integrative omics strategy for deciphering the molecular regulation network in maize roots under chromium exposure
P38	**Xiaojiao Hu** <huxiaojiao@caas.cn>	Analysis of Stalk Fiber Quality of Maize
P39	**Jamila Bernardi** <jamila.bernardi@unicatt.it>	Auxin effects on maize seed transcriptome during the early stages of endosperm development
P40	**Meizhong Luo** <mzluo@mail.hzau.edu.cn>	BAC/BIBAC resources and services for functional and comparative genomics studies of maize
P41	**A. Mark Settles** <settles@ufl.edu>	Bulked segregant analysis (BSA) to map 120 *rough endosperm* (*rgh*) seed mutants from the UniformMu transposon-tagging population
P42	**Zhi Li** <lizhi0001@126.com>	CACTA-like transposable element in *ZmCCT* attenuated photoperiod sensitivity and accelerated the postdomestication spread of maize
P43	**Lili Zhang** <lilizhang0946@163.com>	Characterization and cloning of a *slcd* mutant simultaneously affecting seed and leaf color
P44	**Hangsik Moon** <hangsik.moon@syngenta.com>	Characterization of Maize Genes involved in ABA Signal Transduction
P45	**Yongtao Yu** <yyt0112@hotmail.com>	Characterization of population structure of a set of sweet corn inbreds by developing SNP markers based on SLAF-seq
P46	**Chun Zhang** <spring2007318@163.com>	Cloning and characterization of the male sterile 1 (ms1) gene in maize
P47	**Yaou Shen** <shenyaou@aliyun.com>	Combined small RNA and degradome sequencing reveals microRNA regulation during immature maize embryo dedifferentiation
P48	**Chunhua Mu** <maizesd@163.com>	Construction and Characterization of a Bacterial Artificial Chromosome Library of Maize Inbred Line Qi319
P49	**Xiaoli Xiang** <xiaolixiang2012@gmail.com>	Coordination between methionine storage and cysteine and methionine biosynthesis in maize
P50	**Li Song** <lisong3q@163.com>	Cytological Study and Fine Mapping of the Male Sterile Gene ms6044
P51	**Feng Yu** <yufeng8803@gmail.com>	Deciphering the potential tolerance mechanisms against waterlogging stress at maize seedling stage through physiological study and proteomic technique
P52	**Faqiang Li** <fli32@wisc.edu>	Defining the SUMOylation System in *Zea mays* and its Roles in Stress Protection

Biochemical and Molecular Genetics (Continued)

P53	**Xiaqing Wang** <qingcai427@126.com>	Develop a multi-parent advanced generation inter-cross population for complex quantitative traits dissection in maize
P54	**Xia Zhang** <xzhang554@wisc.edu>	Developmental processes controlling seed size in maize evaluated in the Krug seed size populations and derived inbreds
P55	**Ji Yue Wang** <acute2803764@163.com>	Different Expression Analysis of NADH Dehydrogenase in Maize CMS-C
P56	**Fei Ge** <gefei511@gmail.com>	Different Response to Lead-Stress of line 178、9782 and the F1 generation of 178 and 9782
P57	**Linlin Zheng** <zhenglinlin08@gmail.com>	DNA elements required for the *Bx*-gene expression
P58	**Jiani Yang** <Jianiyang@ufl.edu>	Embryo lethal plastid translation mutants and their genetic suppressors in maize
P59	**Hilde Nelissen** <hilde.nelissen@psb.vib-ugent.be>	Expanding the toolbox to study the dynamics of molecular and cellular processes in the maize leaf growth zone
P60	**He Sun** <ndsh@163.com>	Fine Mapping and Cloning the Unidirectional Cross Incompatibility gene *Ga1-m* in Maize (*Zea mays* L.)
P61	**Guoying Wang** <wangguoying@caas.cn>	Fine mapping of leafy, a dominant mutant conferring extra leaves above ear in maize
P62	**Changzheng Xu** <xucz@swu.edu.cn>	Functional divergence of the paralogous LATERAL ORGAN BOUNDARIES DOMAIN proteins RTCS and RTCL during shoot-borne root development in maize (*Zea Mays* L.)
P63	**Xu Luo** <81999737@qq.com>	Genome Expression Profile Analysis of the Immature Maize Embryo during Dedifferentiation
P64	**Yongzhong Zhang** <yongzz@iastate.edu>	Genome expression profile analysis reveals important transcripts in maize roots responding to the stress of heavy metal Pb
P65	**Orawan Jittham** <oji036@hotmail.com>	Genome-wide association study dissects the genetic architecture of carotenoid biosynthesis in maize kernels
P66	**Chuanxiao Xie** <xiechuanxiao@caas.cn>	Genome-wide identification of miRNAs and their targets in response to low- nitrogen stress in maize
P67	**Guifeng Wang** <holdonhero2000@shu.edu.cn>	Genome-wide identification, splicing and expression analysis of the myosin gene family in maize (*Zea mays*.)
P68	**Suzhi Zhang** <suzhi1026@163.com>	Genome-wide transcriptional profile analysis of Al-responsive genes in maize
P69	**Liuji Wu** <wlj200120@163.com>	Identification and characterization of an E3 ubiquitin ligase Rbx1 in maize (Zea mays L.)
P70	**Suzhen Li** <lisz-1208@163.com>	Identification and characterization of the Zinc-regulated transporters, Iron-regulated transporter-like Protein (ZIP) gene family in maize
P71	**Haijian Lin** <linhj521@gmail.com>	Identification and functional analysis of differentially expressed miRNA in maize (Zea mays L.) in response to Banded Leaf and Sheath Blight

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P72	**Ling Xu** <xu_ling2006@126.com>	Identification of candidates for two major QTLs that confer resistance to gray leaf spot in maize
P73	**Weihua Li** <liwh416@163.com>	Identification of QTL for Starch, protein and Oil content in maize kernel at different filling stages under multi-environments
P74	**Chaolong Lu** <scorpioal@gmail.com>	Identification the reference genes of maize and tesing by qRT-PCR
P75	**Juren Zhang** <jrzhang@sdu.edu.cn>	Improvement of amylose synthesis in maize endosperm through RNAi down-regulating starch branching enzyme IIa and IIb
P76	**Swaran Lata** <slatasharma@gmail.com>	Introgression of QPM trait into non-qpm elite maize inbred lines through marker-assisted breeding
P77	**Kun Wang** <wangkun2538@163.com>	Isolation and functional analysis of ZmPHR genes in maize
P78	**Jun Zhao** <zhaojun01@caas.cn>	Maize NCP1 is an EAR motif-lacking NINJA family protein that negatively regulates drought and ABA responses through interacting with and inhibiting the activity of transcription factor ABP9
P79	**Xiqing Ma** <ma244@purdue.edu>	Mapping novel genes of carbon partitioning using the Maize SNP50 chip
P80	**Jianxin Shi** <sjianxin@gmail.com>	Metabolic map of mature maize kernels
P81	**Jianbing Yan** <yjianbing@gmail.com>	Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights
P82	**Dong Ding** <dingdong0216@hotmail.com>	MicroRNA transcriptomic analysis of heterosis during maize seed germination
P83	**Peng Li** <lp19910301-lp@163.com>	Molecular Characterization of the Restorer-of-Fertility Locus Rf3 of the S-type Cytoplasmic Male Sterility in Maize
P84	**Hua Zhang** <zhanghua@genetics.ac.cn>	Morphological and Transcriptomic Analysis of a Male Sterile Line in Maize (*Zea Mays L.*)
P85	**Gokhan Hacisalihoglu** <gokhan.h@famu.edu>	Natural Variation Associated with Seed Composition Traits to Improve Micronutrient Efficiency
P86	**Lei Wu** <wlei1005@163.com>	Overexpression of the Maize *GRF10*, an Endogenous Truncated GRF Protein, Leads to Reduction in Leaf Size and Plant Height
P87	**Jun Zheng** <zhengjun02@caas.cn>	Overexpression of ZmSnRK2.11, an SNF1 type serine threonine protein kinase in maize, decrease tolerance to osmotic stress in Arabidopsis
P88	**Jonathan Saunders** <jonosaun@ufl.edu>	Phenotype to genotype with “forward-genetic Mu-seq”
P89		Removed from program.
P90	**Jonathan Wang** <jonathan.wang@lifetech.com>	Single reaction multiplex TaqMan assay for simultaneous multiple SNP and CNV data acquisition
P91	**Mo Ju Cao** <caomj@sicau.edu.cn>	Study about the specific fragment accompanied with fertility restored F1 of CMS-C male sterile line in maize(zea mays) by SSR

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P92 **David Braun** <braundm@missouri.edu>
Sucrose Transporter1 function is essential for long-distance transport of sucrose in maize

P93 **Junya Zhang** <zhangjunya@ufl.edu>
The *defective kernel5* (*dek5*) locus encodes a plant specific protein required for grain-fill and chloroplast function

P94 **Kun Li** <likun19880117@126.com>
The genetic architecture of rind penetrometer resistance in two maize recombinant inbred line populations

P95 **Yaqun Huang** <hyqun@hebau.edu.cn>
The genetic architecture of zinc and iron content in maize grains as revealed by QTL mapping and meta-analysis

P96 **Xiaoli Ma** <maxiaoli2011@gmail.com>
The maize glossy26 gene encodes an enoyl-CoA reductase involved in the biosynthesis of epicuticular waxes

P97 **Elisa Gomez** <elisa.gomez@uah.es>
The role of the basal endosperm transfer cells in the protection of the kernel against pathogens

P98 **Yingjia Han** <hanyingjia325@163.com>
The *ZmSAD1* gene is associated with natural variations of stearic acid composition in maize kernels

P99 **Yongjie Liu** <binglinghua66@163.com>
Transcriptome analysis of maize in response to *Fusarium graminearum* infection

P100 **Tingting Guo** <gwt277@126.com>
Ultra-high-density markers reveal the genetic basis of grain yield heterosis in an "immortalized F_2" maize population

P101 **Hai Wang** <wanghai01@caas.cn>
Unraveling the regulatory network of maize defense response to *Ostrinia furnacalis*

P102 **Guihua Lu** <Guihua.lu@pioneer.com>
Use Of Forward Genetic Screens In Rice For Trait Discovery

P103 **Zongliang Chen** <zchen@cau.edu.cn>
Wrinkled kernel1 encodes beta-tubulin5 protein that is required for sister chromatin segregation and endosperm development in maize

P104 **Zhang Zhongbao** <happyzzb@126.com>
X1-homologous genes family as central components in biotic and abiotic stresses response in maize (Zea Mays.L.)

Cell and Developmental Biology

P105 **Michael Muszynski** <mgmuszyn@iastate.edu>
Activation of cytokinin signaling in maize leaves uncovers a potential organogenic expression program at the grass leaf margin

P106 **Jason Nichols** <jason.nichols@syngenta.com>
An automated Whole Plant Phenotyping System for early vigor trait screening and selection

P107 **Dale Brunelle** <dale.brunelle@email.und.edu>
Analysis of maize embryo morphogenesis in nine emb mutants

P108 **Wei Li** <wli@waksman.rutgers.edu>
Barren inflorescence3, a novel semi-dominant maize mutant defective in meristem initiation and maintenance

P109 **Gregorio Hueros** <gregorio.hueros@uah.es>
BETL9 and BETL9like, two genes encoding non-specific lipid transfer proteins with a complementary transcription pattern at the outer surface of the developing maize endosperm

P110 **Philip Becraft** <becraft@iastate.edu>
Brassinosteroid signaling in maize shoot development

P111 **Katie Murphy** <kmurphy3@stanford.edu>
Cell Wall Histochemistry of Maize Anthers

P112 **Jingjuan Yu** <yujj@cau.edu.cn>
Characterization of miRNAs and their targets in maize seed development

P113 **Han Zhang** <zhanghan@stanford.edu>
Convergent Evolution of Maize phasiRNAs and Mammalian piRNAs Supporting Male Reproduction

P114 **Youhui Tian** <tianyouhui@yeah.net>
Cytological Study and Fine Mapping of the Male Sterile Gene ms5 in Maize

P115 **Jinyan Guo** <j.yanguo@gmail.com>
Genetic Interaction between Bract Suppression Genes *tasselsheath1* and *tasselsheath2/4* in Maize

P116 **Clinton Whipple** <whipple@byu.edu>
Genetics and transcriptomics of bract suppression in maize

P117 **M Gerald (Gerry) Neuffer** <gneuffer@gmail.com>
Lesion Mimics, Target Spot: Model for Cell Death Signalling

P118 **Anding Luo** <aluo@uwyo.edu>
Live cell imaging of the receptor like kinase WARTY2 and its role in epidermal patterning of bulliform-like cells during maize leaf development

P119 **Anne Sylvester** <annesyl@uwyo.edu>
Maize Cell Genomics: Developing a two component transactivation system

P120 **Kin Lau** <lau3@purdue.edu>
Mapping and Characterization of the Maize Mutant, *Clumped tassel1* (*Clt1*)

P121 **Sivamani Elumalai** <sivamani.elumalai@syngenta.com>
Method development for an elite maize line callus transformation thru agrobacterium

P122 **Zhaoxia Li** <zhaoxia_1019@126.com>
miRNA alterations are an important mechanism in the adaptation of maize to a low phosphate environment

P123 **Dabing Zhang** <zhangdb@sjtu.edu.cn>
Molecular Control of Grass Inflorescence Development

P124 **Guo-Ling Nan** <gnan@stanford.edu>
Ms23, a bHLH transcriptional factor, regulating the tapetal cell fate in pre-meiotic anther of maize

Cell and Developmental Biology (Continued)

P125 **Matt Evans** <mevans@carnegiescience.edu>
Mutant analysis of maize antipodal cells and auxin signaling

P126 **Liwei Liu** <liuliwei89@yeah.net>
Preliminary Study of Polyembryonic Seedling in Maternal Haploid Induction

P127 **Rachel Egger** <egger@stanford.edu>
Quantifying maize tassel development and correlating tassel length with anther development

P128 **Peter Rogowsky** <peter.rogowsky@ens-lyon.fr>
Regulation of maize kernel filling: the role of *ZmZOU* in embryo-endosperm communication

P129 **Johanna Smyth** <smythj@science.oregonstate.edu>
Teasing out the transcriptome of *in vivo* germinated pollen

P130 **Hong Yao** <yaoho@missouri.edu>
The *barren stalk2* Gene Is Required for Axillary Meristem Development in Maize

P131 **Yura Goncharov** <wild91@list.ru>
Types of Morphogenesis in Callus Tissue oF Lancaster Maize Group

P132 **Jose Dinneny** <jdinneny@carnegiescience.edu>
Water acts as a positional signal to pattern root architecture in maize

P133 **Jingjuan Yu** <yujj@cau.edu.cn>
Zm908p11, encoded by a short open reading frame (sORF) gene, functions in pollen tube growth as a profilin ligand in maize

P134 **Zhaobin Dong** <zbdong@cau.edu.cn>
ZmLAZY1 regulates shoot gravitropism and inflorescence development through polar auxin transport and auxin signaling

Cytogenetics

P135 **Jing Zhang** <zhangjing@genetics.ac.cn>
A novel central element of the synaptonemal complex is required for centromere pairing in maize

P136 **Handong Su** <shdong@genetics.ac.cn>
Bub1 and Bub3 Play An Important Role in Plant Chromosome Orientation and Separation

P137 **Yalin Liu** <ylliu@genetics.ac.cn>
Centromere changes in progeny of misdivision

P138 **Yaping Yuan** <yuanyp@jlu.edu.cn>
Differential gene expression of maize (Zea mays L.) inbred line H99 during somatic embryogenesis

P139 **Bing Zhang** <bzhang@genetics.ac.cn>
Identification and Characterization of ZmHaspin kinase as an essential kinase during cell division

P140 **Dongwei Guo** <gdwei@nwsuaf.edu.cn>
Isolation and Flow Purification of Endosperm Protoplast in Developing Seed of Maize

P141 **Eduard KHatefov** <haed1967@rambler.ru>
Problem of seed fertility of tetraploid corn and possible ways of its decision

P142 **Meng Yujie** <mengyujie25@126.com>
The Regeneration System of a High Oil Maize (GY302)

Education & Outreach

P143 **Carson Andorf** <carson.andorf@ars.usda.gov> — Chinese Translation of MaizeGDB and the Maize Genetics Conference websites

P144 **Jack Gardiner** <jack.m.gardiner@gmail.com> — Education and Outreach at MaizeGDB

P145 **Darwin Campbell** <dawin.campbell@ars.usda.gov> — Evolution of the Maize Research Community

P146 **Mary Schaeffer** <mary.schaeffer@ars.usda.gov> — New avenues to contribute your data to MaizeGDB: *gene functions, genome assembly problems, maize gene wiki*

P147 **Jason Williams** <williams@cshl.edu> — DNA Subway: A Simple, Powerful Bioinformatics Workflow for RNA-Seq Analysis and Distributed Genome Annotation

P148 **Denise Costich** <d.costich@cgiar.org> — The CIMMYT Maize Lines (CMLs): A critical genetic resource for maize breeders and geneticists

P149 **Denise Costich** <d.costich@cgiar.org> — The Maize Germplasm Bank at CIMMYT: Global source of maize genetic resources

P150 **Michael Oke** <maof2020@gmail.com> — The Over view of a Typical Maize waste products usage in Gwagalada Area Council of the Federal Capital Territory

Quantitative Genetics & Breeding

P151 **Qingqing Liu** <677qing@163.com> — A molecular chaperone ZmTRXh confers resistance to sugarcane mosaic virus (SCMV) in maize

P152 **Yang Zhao** <zhaoyang0521@163.com> — A Novel Maize Homeodomain-leucine Zipper (HD-Zip) I Gene, Zmhdz10, Positively Regulates Drought and Salt tolerance in both Rice and Arabidopsis

P153 **Zhongyi Wu** <zwu22@126.com> — A simple, novel and high efficiency transformation method to introduce foreign DNA into corn plants

P154 **Alexander Lipka** <AEL54@cornell.edu> — A Systems Approach to Identify Genetic Control of Tocochromanol Variation in Maize Grain

P155 **Valeriu Rotarenco** <valeriu.rotarenco@procera.ro> — Advancing the production of maize haploids in-vivo

P156 **Thomas Lubberstedt** <THOMASL@iastate.edu> — Analysis of maize (Zea mays L.) seedling roots with a new high-throughput tool to connect seedling roots to adult roots

P157 **Gaoke Li** <ligaoke790326@163.com> — Analysis of the Causal Factors and Degree of Heterosis in the Haploid Induction Ratio in Maize

P158 **Junping Chen** <junping.chen@ars.usda.gov> — Association Study of High Temperature Tolerance Traits in Maize

P159 **Pengfei Leng** <pfleng@iastate.edu> — Characterization of the Sugarcane Mosaic Virus *Scmv2* Resistance region by Positional Association analysis in Maize

P160 **Huafeng Chen** <chenhuafengchf@163.com> — Characterize the genetic basis of maize leaf traits using a large maize-teosinte population

P161 **Bailin Li** <Bailin.Li@cgr.dupont.com> — Cloning and Characterization of a QTL for salinity tolerance in maize

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P162 **Baobao Wang** <wangbao.com.cn@163.com>
Combining large recombinant inbred lines and ultra-high density linkage map to dissect the plant architecture traits in maize

P163 **Yongfu Tao** <blank_tao@163.com>
Discovery of a candidate gene underlying the QTL-*qMrdd1* that confers recessive resistance to maize rough dwarf disease

P164 **Dingyi Xu** <minen1134@163.com>
Dissect the genetic basis of maize inflorescence domestication

P165 **Jie Liu** <liujiedp@gmail.com>
Dissection the genetic basis of maize kernel weight and shape

P166 **Yuncai Lu** <luyuncai@hlju.edu.cn>
Emergence under chilling stress of maize inbred lines by Genome Wide Association Study (GWAS)

P167 **Haochuan Li** <Lihaochuan1220@163.com>
Evaluation of inducing parthenogenesis ability for different inducers in maize

P168 **Shutu Xu** <Shutuxu@nwafu.edu.cn>
Expression analysis of chlorophyll-degrading related genes during dark-induced leaf senescence in maize

P169 **Yilin Cai** <caiyilin1789@163.com>
Fine mapping of *qAC6* and epistasis analyzing with *qAC10* controlling anthocyanin biosynthesis of kernel in maize

P170 **Lei Liu** <leil@webmail.hzau.edu.cn>
Fine-mapping and Cloning a Major Effect Kernerl Row Number QTL *qKRN4e* in Maize

P171 **Xingming Fan** <xingmingfan@163.com>
Generation mean analysis for grain yield among three maize heterotic groups

P172 **Yongzhong Zhang** <yongzz@iastate.edu>
Genetic and agronomic assessment of cob traits in corn under low and normal nitrogen management conditions

P173 **Cheng Huang** <chenghuang2014@163.com>
Genetic Architecture of Vascular Bundle In Stem During Maize Domestication

P174 **Pengcheng Li** <lipc20052006@163.com>
Genetic association of nitrogen use efficiency and root-related traits in a RIL population of maize

P175 **Weiya Li** <liweiya66@126.com>
Genetic control of leaf width variation by *Cellulose Synthase-Like D1*

P176 **Lin Zhang** <zhanglin@sibs.ac.cn>
Genetic dissection and fine mapping of rice QTLs for panicle length and evaluation of their potential to increase yield

P177 **Hong Wang** <hongwangfromchina@gmail.com>
Genetic dissection of tocopherol concentration in maize kernel combining linkage and association analyses

P178 **Ning Yang** <yangningyingji@126.com>
Genome Wide Association Studies using a new nonparametric model reveal the genetic architecture of 17 agronomic traits in an enlarged maize association panel

P179 **Hongjun Liu** <lhj20305@163.com>
Genome wide genetic marker discovery and genotyping using next-generation sequencing in maize IBM Syn10 mapping population

P180 **Sheng Xue Liu** <liushengxue1983@163.com>
Genome-Wide Analysis of *ZmDREB* Genes and Their Association with Natural Variation in Drought Tolerance at Seedling Stage of *Zea mays* L.

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P181 **J Alberto Romero Navarro** <jar547@cornell.edu>
Genome-wide association and meta-analysis on a comprehensive panel of 4,500 maize accessions from CIMMYT's breeder's core collection

P182 **Min Deng** <hdengmin@163.com>
Genome-Wide Association Study and Linkage Mapping dissect the Genetic Architectures of Amino Acids in Maize Kernel

P183 **Yingjie Xiao** <shanren0179@gmail.com>
Genome-wide dissection of maize ear traits by multiple-parents population based linkage and association analyses

P184 **Jin Liu** <lj-9000@163.com>
Grain moisture measurement based on Low-Field Nuclear Magnetic Resonance in maize (*Zea mays L.*)

P185 **Peter Balint-Kurti** <pjbalint@ncsu.edu>
How corn got its spots: the genetics of lesions, disease and the defense response

P186 **Shimin Liu** <liushimin5118@163.com>
Identification of Disease Resistance to Maize Rough Dwarf Virus in Core Inbred Lines

P187 **Matthieu Falque** <falque@moulon.inra.fr>
Intraspecific variation of recombination rate in Maize

P188 **Hongwei Zhang** <youthzhw@163.com>
Large-scale germplasm evaluation and candidate gene identification for low phosphorus tolerance in maize

P189 **Dandan Wu** <usual_1988@163.com>
Mapping QTLs for Salt Tolerance Based on SNP Markers at Seedling Stage in Maize (*Zea Mays L.*)

P190 **Xin Dong** <dongxinnx53@163.com>
Marker assisted selection of high oil in vivo haploid inducers in maize

P191 **Jianbing Yan** <yjianbing@mail.hzau.edu.cn>
Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights

P192 **Renyu Zhang** <zhangrenyu@live.com>
Patterns of Genomic Variation in Chinese Maize Inbred Lines and Implications for Genetic Improvement

P193 **Songlin Hu** <hsonglin@iastate.edu>
Phenotype-selected introgression library for studying genetic architecture of plant height in maize

P194 **Odunayo Olawuyi** <olawuyiodunayo@yahoo.com>
Potentials of Arbuscular Mycorrhiza fungus Gigaspora gigantea in tolerating drought in maize (Zea mays L.)

P195 **Yuling Li** <yuling_li@126.com>
QTL detection for grain water relations and genetic correlations with grain matter accumulation at four stages after pollination in maize

P196 **Yongbin Dong** <dyb0816@163.com>
QTL Mapping and Meta-analysis for Kernel Composition Traits across Three Generations in Popcorn

P197 **Jiaojiao Ren** <renjiaojiao789@sina.com>
QTL mapping for haploid male fertility in maize (Zea mays L.)

P198 **Eduard Khatefov** <xinfangliu2002@126.com>
Research for Autotetraploid Corn

P199 **Valeriu Rotarenco** <valeriu.rotarenco@procera.ro>
Selection at the level of haploid sporophyte as an efficient tool in maize improvement

P200 **Soon-Kwon Kim** <soonkwonkim@gmail.com>
Six-Year Experiences of Genetic Vulnerability of Maize Hybrids in China

P201 **Bingde Dou** <doubd@163.com>
Sweet corn germplasm innovation for wine-making and its' application in breeding

Transposons & Epigenetics

P202 **Yubin Li** <yubin@waksman.rutgers.edu> — A user-friendly sequence-indexed reverse genetics resource for maize

P203 **Charles Hunter** <ibe@ufl.edu> — Activity and evolution of non-canonical Mutator transposons in maize

P204 **Zhenling Lv** <luzl015@genetics.ac.cn> — Analysis of gene transcription and DNA methylation within the Dp3a neocentromere

P205 **Fang Bai** <fbai001@ufl.edu> — Analysis of imprinted genes with developmental functions in the maize seed

P206 **Agata Tyczewska** <agatat@ibch.poznan.pl> — Changes in DNA methylation profile in maize under herbicide stress conditions

P207 **Miaoyun Xu** <xumiaoyun@caas.cn> — Characterization of miR169 and NF-YA families and their expression pattern responding to abiotic stresses in maize

P208 **Miriam N Salazar-Vidal** <msalazar@langebio.cinvestav.mx> — Characterization of the role of duplicated *ZmPho1;2* genes in maize phosphate homeostasis

P209 **Amanda Waters** <water157@umn.edu> — Comprehensive analysis of imprinted genes in maize reveals allelic variation for imprinting and limited conservation with other species

P210 **Alice Lunardon** <alice.lunardon@studenti.unipd.it> — *De novo* small RNA loci annotation in maize leaves and their expression changes in response to environmental stresses

P211 **Shaojun Xie** <xieshaojun0621@cau.edu.cn> — Epigenetic variation through the breeding processes of maize

P212 **Cristian Forestan** <cristian.forestan@unipd.it> — Epigenetics regulation of maize transcriptome: from coding, *via* TEs, to long non-coding RNAs and beyond

P213 **Cristian Forestan** <cristian.forestan@unipd.it> — Genetic and epigenetic regulation of response to drought and salt stress in maize

P214 **Zhen Liu** <liuzhen521000@126.com> — Genome-wide analysis of terminal-repeat retrotransposons in miniature (TRIM) in maize

P215 **M Gerald (Gerry) Neuffer** <gneuffer@gmail.com> — Guide to Maize Mutant Phenotypes (http://mutants.maizegdb.org)

P216 **Gernot Presting** <gernot@hawaii.edu> — Rapid invasion of euchromatic regions by centromeric retrotransposons at sites of neocentromere formation in maize

P217 **Ning Jiang** <jiangn@msu.edu> — Selective acquisition and retention of genomic sequences by Pack-MULEs in grasses

P218 **Tzuu-fen Lee** <tzuufen@dbi.udel.edu> — Small RNA profiling revealed the maize *Ufo1*-modulated epigenetic regulation via 24 nt small RNA in tissue-specific manner

P219 **Maike Stam** <m.e.stam@uva.nl> — Specific tandem repeats are sufficient for paramutation-induced trans-generational silencing

P220 **Zuxin Zhang** <zuxinzhang@mail.hzau.edu.cn> — ZmAGo18a, a meiosis-enriched Argonaute, is required for inflorescence development and epigenomic reprogramming of the male gametophyte in maize

P221 **Donya Shodja** <dnshodja@oakland.edu> — RNA Binding Protein 48 (RBM48) is Critical for Maize Endosperm Development and Plant Viability

Plenary Talk Abstracts

Plenary 1 **Thursday, March 13 7:15PM**

A systems approach to understanding photosynthetic differentiation in the grasses

(presented by Tom Brutnell <tbrutnell@danforthcenter.org>)
Full Author List: Brutnell, Tom[1,2], Ahern, Kevin[3], Anderson, Tim[1], Li, Pinghua[4], Mertz, Rachel[5], Studer, Tony[1], Wang, Lin[6], Weissmann, Sarit[1] ;
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C_4 photosynthesis is utilized by over 60 lineages of land plants to fix carbon and has evolved multiple, independent times in the angiosperms. In the grasses alone C_4 has evolved over 17 times independently and is dependent on the metabolic cooperation of two specialized cell types, the bundle sheath and the mesophyll. In maize, carbon is fixed in the mesophyll as a C_4 acid, either as malate or aspartate, that then diffuses to the bundle sheath where it is decarboxylated releasing CO_2 in the vicinity of Rubisco. This CO_2 pump results in high local CO_2 concentrations in the BS plastids that effectively eliminates photorespiration. As photorespiration can reduce photosynthetic capacity of C_3 plants by as much as 30%, C_4 plants are at a competitive advantage under hot dry conditions when photorespiration is prevalent in C_3 plants. A deeper understanding of the genes and networks underlying C_4 photosynthetic differentiation will provide new opportunities for breeding improved varieties of C_4 crops and for the engineering of C_4 traits into C_3 grasses.

We have begun the functional dissection of C_4 photosynthesis by exploiting the excellent genetic and genomic resources available in maize including transposon collections and RNAseq expression profiling. I will present the findings of our recent genetic studies to define the components of the C_4 carbon concentrating mechanism and present preliminary studies into the use of a new model system, *Setaria viridis*, to accelerate gene discovery in the grasses and begin the engineering of C_4 pathways.

Understanding the molecular basis of rice tillering

(presented by Jiayang Li[1] <jyli@genetics.ac.cn>)
[1] State Key Laboratory of Plant Genomics and National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Plant architecture is a primary determinant of crop yield and has profound adaptability in response to internal and external factors. The pattern and timing of shoot branching, or tillering in monocot crops, determine overall biomass and ultimate yield of crops, which are basically result from the coordination of the internal developmental program with external environmental stimuli. Tremendous efforts have been made, showing that plant hormones and key transcription factors play critical roles in regulating the developmental flexibility of plant architecture. However, plant hormonal regulation of shoot branching is complex and far from understood.

We have identified and characterized a series of rice mutants that show various altered plant tillering phenotypes. The corresponding genes were cloned by map-based cloning approaches. It has been demonstrated that the tillering phenotypes are mainly resulted from either the deficiency in the branching plant hormone biosynthesis and signaling or the key transcription factors. Further dissection of the functions of these genes revealed their critical roles in the regulation of plant architecture. Our work allows us to further understand the molecular mechanisms underlying hormone action and functions of key transcription factors, which will contribute to the rational application of plant growth regulators. With the increased knowledge of the factors controlling the plant architecture of rice, it becomes possible to breed higher-yield elite varieties by manipulating the genetic network that determines rice plant architecture.

This work was supported by Grants from National Natural Science Foundation of China, Ministry of Science and technology of China and Chinese Academy of Sciences.

Molecular breeding for complex adaptive traits – how integrating crop ecophysiology and modelling can enhance efficiency

(presented by Graeme Hammer[1] <g.hammer@uq.edu.au>)
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Progress in crop improvement is limited by the ability to identify favourable combinations of genotypes (G) and management practices (M) in relevant target environments (E) given the resources available to search among possible combinations. Phenotypic performance of the array of possible combinations forms what can be viewed as an adaptation landscape. Crop improvement then becomes a search strategy on that complex G*M*E landscape. However, currently we cannot reliably predict (and navigate to) the desired destination on the adaptation landscape. We require prediction of phenotype based on genotype to underpin yield advance. In plant breeding, traditional methods have involved measuring phenotypic performance (yield) of large segregating populations in multi-environment trials and applying rigorous statistical procedures based on quantitative genetic theory to identify superior individuals. This phenotypic selection approach has been successful but inefficient. Developments in molecular genetic technologies have allowed the focus of practical crop improvement to shift from the level of the individual (genotype) to the level of genomic region. The ability to inexpensively and densely map/sequence genomes has facilitated development of molecular breeding strategies using genome wide prediction approaches. However, their applicability to complex traits also remains constrained by gene-gene and gene-environment interactions, which restrict the predictive power of associations of regions with phenotypic responses. Despite the limitations of such context dependencies, it has been possible to design molecular breeding strategies for complex traits that on average outperform phenotypic selection.

Here it is argued that crop ecophysiology and functional whole plant modelling can provide an effective link between molecular and organism scales to enhance efficiency of molecular breeding approaches. A quantitative physiological framework that facilitates robust dissection of complex traits can inform trait targets for phenotyping, mapping, and genomic prediction in a manner that reduces context dependencies. Plant/crop modelling underpins prediction of likely phenotypic consequences of molecular breeding in target environments. This approach holds considerable promise for effectively linking genotype to phenotype for complex adaptive traits and thus enhancing efficiency of molecular breeding. In this presentation, specific examples are presented for drought adaptation in sorghum and maize.

Polyploidy in maize and its relatives

(presented by Toby Kellogg <ekellogg@danforthcenter.org>)
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Polypoidy, particularly allopolyploidy, is common in flowering plants and is thought to drive diversification. However, other evidence suggests that polyploidy is instead an evolutionary dead end, and that polyploids do not persist long in evolutionary time. The grass tribe Andropogoneae (sub-family Panicoideae, family Poaceae) contains a large number of polyploids and provides a good system in which to test the putative correlation between polypoidy and diversification. The tribe is a morphologically diverse clade of about 1200 species that contains some of our most economically important taxa (maize (*Zea*), *Sorghum*, and sugarcane (*Saccharum*)). Previous work has suggested a rapid radiation within the tribe and many examples of hybridization are known. We find evidence for frequent allopolyploidization events, most of which do not lead to extensive diversification, indicating that the polyploidization frequently does not lead to diversification. A few older clades, however, exhibit complex histories of crossing and genome doubling. These include the *Zea*/*Tripsacum* group, in which divergence of genera occurred after polyploidization.

McClintock Prize Abstract

McClintock Prize 1 **Friday, March 14 7:00PM**

When genomes meet – RNA, epigenetics and phenotypes of hybrid plant

(presented by David Baulcombe <dcb40@cam.ac.uk>)
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Eukaryotes contain small regulatory RNAs that have been referred to as the dark matter of genetics. They are typically 21-24 nucleotides long, associated with Argonaut or Piwi proteins. Some of these small RNAs guide the Argonaut/Piwi protein to a complementary RNA and they are negative regulators of gene expression acting at the level of messenger RNA turnover or translation. Others participate in more complex epigenetic systems affecting chromatin or they act as part of an RNA signal that moves between cells. In plants the posttranscriptional mechanism is involved in defense against RNA viruses. The chromatin effects play a role in defense against DNA viruses and transposable elements and it is associated with the establishment of heritable epigenetic marks.

However the importance of this defense system goes beyond suppression of transposons and viruses. There are secondary effects of the epigenetic marks that may influence the expression of adjacent genes in the sense of McClintocks “controlling elements”. In most instances the effect is gene silencing and in some instances the effect may influence the biology of the affected plant. I will describe how RNA silencing may be particularly important following wide cross hybridisation and how it may influence hybrid vigour and transgressive segregation.

Short Talk Abstracts

SESSION 2 - DEVELOPMENTAL GENETICS, QUANTITATIVE GENETICS I
Chair: Peter Balint-Kurti Friday, March 14. 8:15 AM – 9:55 AM

T1

The SPB-box transcription factors *unbranched2* and *unbranched3* function redundantly with *tasselsheath4* to regulate plastochron index

(presented by George Chuck <georgechuck@berkeley.edu>)
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Maintenance of an apical meristem requires tight regulation of the balance between losing cells to lateral primordia versus gaining cells through division. If the rate of lateral primordia initiation is not regulated, too many primordia may be initiated at the expense of the meristem. We recently discovered that mutations in members of the *tasselsheath4 (tsh4)* clade of SBP-box transcription factors play a major role in regulating this process. A reverse genetic screen performed with genes similar to *tsh4* uncovered two duplicate loci, *unbranched2 (ub2)* and *unbranched3 (ub3)*, which abolish tassel branch initiation in double mutant combinations. The unbranched phenotype is enhanced by the presence of the *tsh4* mutation. In addition, double and triple mutant combinations with *tsh4* revealed that these genes also play a role in restricting leaf initiation as well as tiller number. SEMs of *ub2/ub3* double mutant tassels revealed an excess of lateral primordia that initiate at a high rate, greatly reducing the size of the inflorescence meristem. An antibody was raised to the UB2 and 3 proteins and used for immunolocalization. UB2 and 3 proteins were found throughout the lateral domains of the meristem and leaf primordia, but were absent from the central domain of the meristem where regeneration occurs. We hypothesize that *ub2, ub3* and *tsh4* are functionally redundant factors necessary for controlling cell partitioning to lateral domains of the meristem. In their absence, cells are allocated to lateral primordia at a higher rate, leaving fewer cells within the central domain of the meristem available for regeneration. Interestingly, *ub3* associates strongly with tassel branch and ear row number QTL in the NAM population, indicating that these genes are likely to be agronomically important

Funding acknowledgement: Department of Energy (DOE)

T2

SWEET transporters and seed filling: tools for plants or opportunities for pathogens?

(presented by Davide Sosso <dsosso@stanford.edu>)
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All life requires energy to develop and reproduce. Through photosynthesis plants transform sunlight energy and store it as sugars or macromolecular carbohydrates. Sugars are the dominant transport form of carbon in plants. In maize, despite its great importance for yield improvement and plant vigor, the regulation of and mechanism of sugar flow from sources (leaves) to sinks (roots, flowers, seeds) are poorly understood parts of the energy conversion chain. Recently, we identified the elusive sugar transporters (SWEET) responsible for the first step of this sugar flow (leaf phloem loading); more recent unpublished data suggest that a set of SWEETs is also critical for the last step (seed filling) and we present here our preliminary findings. Sugar flow is likely under tight control to optimize both allocation and prevent any losses. Many different pathogenic species attack and feed on plants, creating massive yield losses. Pathogens have evolved strategies to gain access to plant metabolites, specifically by recruiting SWEETs to divert sugar flow towards the site of infection. SWEET sugar transporters had previously been implicated as targets of blight bacteria in rice, and when induction of SWEETs is blocked, plants are resistant to the pathogen, with exciting implications for agriculture. In maize, a fungal pathogen (*U. maydis*) invades stems, leaves, and flowers and triggers tumor formation (i.e. infected maize seeds are converted into tumors 50 to 100x bigger than normal seeds). These tumors are a novel aggressive sink tissue that, by manipulating SWEET expression, induces release of host sugars to feed the pathogen and permit completion of spore formation. With the goals of visualizing sugar efflux and quantifying when and where sugars are released in the vicinity of the fungal hypae, we deployed our novel sugar FRET nanosensors both in the pathogen and maize.

Funding acknowledgement: United States Department of Agriculture (USDA), Stanford University Bio-X Initiative

T3

Auxin signaling in the early steps of maize inflorescence development

(presented by Andrea Gallavotti <agallavotti@waksman.rutgers.edu>)
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The initiation of axillary meristems is a crucial process occurring throughout plant development and ultimately drives the variation in plant architecture observed among different species. In maize inflorescences, axillary meristems are continuously initiated at the flanks of inflorescence meristems, and eventually result in the formation of long branches, spikelets and flowers. Analyses of different maize mutants affected in branch, spikelet and flower formation have revealed the essential role of auxin in this developmental process. Mutations altering auxin biosynthesis, transport and signaling all cause defects in the initiation of axillary meristems. We have characterized two semi-dominant maize mutants, *Barren inflorescence 1* and *4*, that are severely impaired in the initiation of axillary meristems. We cloned the *bif1* and *bif4* genes using a candidate gene approach and showed that in both cases the affected loci encode previously uncharacterized Aux/IAA transcriptional regulators involved in auxin signaling. Auxin signal transduction is a relatively short pathway that is driven by the rapid release of Aux/IAA-mediated negative regulation via auxin-induced protein degradation. BIF1 and BIF4 are part of repressor complexes that suppress transcription of target genes during axillary meristem initiation. Using a combination of genetic and molecular approaches we are identifying upstream and downstream components of this pathway. We will present an integrated model for how auxin functions in post-embryonic meristem initiation and how BIF1 and BIF4 contribute to this essential developmental process.

Funding acknowledgement: National Science Foundation (NSF)

T4

The B-class mutant *sterile tassel silky ear1* (*sts1*) provides a window into both conserved and divergent aspects of maize floral development

(presented by Madelaine Bartlett <mbartlett@bio.umass.edu>)
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The B-class MADS box genes have been repeatedly identified as key regulators of second and third floral whorl organ identity in a number of divergent flowering plant lineages. We show that in maize, despite distinctive floral morphology, the B-class gene *sterile tassel silky ear1* (*sts1*) also controls the development of second and third whorl floral organs. Characterization of the *sts1* mutant has revealed conserved, convergent, and divergent aspects of B-class function in maize. We show that the B-class proteins of maize bind DNA as obligate heterodimers that regulate their own expression, as is the case in *Arabidopsis* and *Antirrhinum*. Surprisingly, this obligate heterodimerization, coupled to autoregulation, appears to have evolved convergently in the order that contains the grasses. The *sts1* mutant phenotype is unusual in some aspects, relating in particular to sex determination. Upon further investigation, these unusual aspects helped to reveal that carpel abortion in the maize tassel occurs in an organ-specific, rather than in a whorl-specific manner, regulated by *grassy tillers1* (*gt1*). STS1-YFP localization patterns also show some features particular to maize. Taken together, these results help illuminate both the conserved and the novel attributes of gene function that underlie morphological diversity.

Funding acknowledgement: National Science Foundation (NSF)

T5

Transcriptomes and Proteomes Define Gene Expression Progression in Pre-meiotic Maize Anthers

(presented by Rachel Egger <egger@stanford.edu>)
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Plants lack a germ line, consequently during reproduction adult somatic cells within flowers must switch from mitotic proliferation to meiosis. In maize (Zea mays L.) anthers, hypoxic conditions in the developing tassel trigger pre-meiotic competence in the column of pluripotent progenitor cells in the center of anther lobes, and within 24 hours these newly specified germinal cells have patterned their surrounding neighbors to differentiate as somatic niche cells. Transcriptomes were analyzed by microarray hybridization in carefully staged whole anthers during initial specification events, after the separation of germinal and somatic lineages, during the subsequent rapid mitotic proliferation phase, and during final pre-meiotic and somatic cell differentiation. Maize anthers exhibit a highly complex transcriptome constituting nearly three quarters of annotated maize genes, and expression patterns are dynamic. Laser microdissection was applied to begin assigning transcripts to tissue and cell types and for comparison to transcriptomes of mutants defective in cell fate specification. Whole anther proteomes were analyzed at three developmental stages after mass spectrometric peptide sequencing on size-fractionated proteins to evaluate the timing of protein accumulation relative to transcript abundance. New insights include early and sustained expression of meiosis-associated genes, an extremely large change in transcript abundances and types a few days prior to meiosis, and the relative disparity between transcript abundance and protein abundance at any one developmental stage.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), American Society of Plant Biologists

T6

Small kernel1 encodes a pentatricopeptide repeat protein required for mitochondrial *nad7* transcript editing and seed development in maize and rice

(presented by Bao-Cai Tan <bctan@sdu.edu.cn>)
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RNA editing modifies cytidines (C) to uridines (U) at specific sites in the transcripts of both mitochondria and plastids, altering the amino acid specified by the DNA sequence. However, the biological significance and the mechanism are not fully understood. Here we report the identification of an editing factor via molecular characterization of a small kernel mutant in maize, *smk1*. Loss of *Smk1* function arrests both the embryo and endosperm development. *Smk1* encodes an E-subclass pentatricopeptide repeat (PPR) protein that is targeted to mitochondria. Comparative analysis of the mitochondrial transcripts revealed that loss of the SMK1 function abolishes the C-to-U editing at *nad7*-836 site, causing a change from leucine to proline. The *smk1* mutant showed dramatically reduced complex I assembly and NADH dehydrogenase activity, increased alternative oxidase 2 (*Aox2*) expression, and abnormal biogenesis of the mitochondria. Analysis of the ortholog in rice (Oryza sativa) revealed that the rice SMK1 has a conserved function in C-to-U editing of the mitochondrial *nad7*-836 site. T-DNA knockout mutants showed abnormal embryo and endosperm development, resulting in embryo or seedling lethality. The leucine at NAD7-279 residue is highly conserved from bacteria to flowering plants, and analysis of the mitochondrial *nad7* genes and SMK1 homologs reveals a molecular co-evolution between the requirement of C-to-U editing and the existence of a SMK1 homolog. These results demonstrate that *Smk1* encodes a PPR-E protein that is required for *nad7*-836 editing, and this editing is critical to NAD7 function in complex I assembly in mitochondria and hence to the embryo and endosperm development in maize and rice.

Funding acknowledgement: Research Grants Council of the Hong Kong Special Administrative Region to B.-C.T. (Project no. 473512 and 473611), and a grant from the National Natural Science Foundation of China to B.-C.T. (Project No. 31170298).

T7

Regulatory networks controlling maize inflorescence architecture and the interface with early season drought responses

(presented by Andrea Eveland <eveland@cshl.edu>)
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Inflorescence architecture is a primary determinant of yield, regulating seed number and harvesting ability, yet the underlying molecular networks remain largely unexplored in the world's most important cereal crops. Here, we use a systems-level approach to elucidate the gene networks that modulate meristem determinacy and inflorescence architecture in maize. Our strategy integrates spatiotemporal expression signatures (i.e. mRNA-seq-based profiles) with morphological changes resulting from genetic perturbations that disrupt discrete steps in the RAMOSA (RA) inflorescence branching pathway. Our network analyses defined distinct developmental modules that contribute to identity and determinacy of grass-specific meristem types, and which appear to have been co-opted from other developmental programs, along with uncharacterized and/or lineage-specific genes. Using ChIP-seq, we also showed that RA1, a C2H2 transcription factor, can both activate or repress genes during development depending on spatiotemporal context. Notably, RA1 repressed expression of the *liguleless1* (*lg1*) gene, restricting its expression to the base of indeterminate, but not determinate, axillary meristems in the inflorescence. Since expression of RA1 is specific to inflorescences, differential regulation of a *liguleless* module co-opted for regulation of tassel architecture traits may underlie divergence from previously known roles in leaf angle.

To place our meristem determinacy network within the larger context of maize inflorescence development, we integrated combinatorial ChIP-seq profiles from KNOTTED1 (KN1) and FASCIATED EAR4 (FEA4), key regulators of meristem maintenance and meristem size, respectively, and identified convergence points in modulation of specific developmental, hormone and signaling networks. We further used our integrated network to define points of interface between development and stress-response networks by incorporating expression signatures from drought-stressed maize inflorescence primordia. Our results provide insightful links between developmental transitions, source-sink regulation and flowering time in response to environmental perturbation, and reflect network control of phenotypic plasticity in grass inflorescence development.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T8

DSY2 is required for meiotic recombination and synaptonemal complex formation

(presented by Chung-Ju Rachel Wang <rwang@gate.sinica.edu.tw>)
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Meiosis is the specific type of cell division required for generating haploid gametes. It contains single round of DNA replication, followed by two rounds of chromosome segregation. Meiotic recombination initiated by double-strand breaks (DSBs) are essential for homologous pairing and the proper segregation of homologues at metaphase I. Synapsis, a process of synaptonemal complex (SC) formation between newly paired homologous chromosomes, promotes meiotic recombination. However, genes required for recombination and synapsis, as well as coordination of both events remain unclear. Maize desynaptic2 (dsy2) mutant exhibits homologous pairing defects, leading to male sterility. We identified that dsy2 gene encodes a coiled-coil protein. Analyses of dsy2 mutant revealed that meiotic recombination and SC formation are defective. We found that DSB formation, the earliest step of recombination, is largely reduced, and the loading of ZmRAD51, a recombinase protein, is also affected in the dsy2 mutant. Super-resolution microscopy showed that DSY2 is located on axial element of SC. Dual immunolocalization studies with other SC proteins showed that DSY2 and ZmASY1, a yeast HOP1 homologue, form distinct alternate pattern along chromosomes in the wild-type. The axial organization of ZmASY1 is slightly impaired in the dsy2 mutant. More importantly, the assembly of mature SC by installing ZmZYP1 protein between axial elements is abolished in the dsy2 mutant. Yeast two hybrid experiments further revealed that ZmZYP1 interacts with DSY2 but does not interact with ZmASY1. This indicates that DSY2 is directly required for the SC formation. Taken the fact that DSY2 also plays a role in DSB formation, we proposed that DSY2 acts as a mediator to coordinate meiotic recombination and synapsis.

T9

The distribution of recombination breakpoints and their association with genomic features in two maize NAM populations

(presented by Peter Bradbury <pjb39@cornell.edu>)
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Nested association mapping (NAM) populations have become a popular tool for combining the power of linkage mapping with the resolution of association analysis. Genotyping by sequencing (GBS), another popular tool for genetics and breeding, provides a low-cost method of generating a large number of molecular markers. We describe the application of GBS to two large maize NAM populations, the US-NAM population, consisting of 25 bi-parental families with B73 as the common parent, and the CN-NAM population, consisting of 11 bi-parental families with Huangzaosi as the common parent. Low-coverage GBS data was used to identify the location of over 137,500 recombination breakpoints with high resolution in a total of 6079 RILs. While the data shows that the distribution of breakpoints is remarkably similar between families and populations, it also identifies locations where cross-over frequency differs significantly. In addition, the large number of breakpoints provides an opportunity to examine the association of cross-overs with a variety of genomic features. Breakpoint locations are compared to the distribution of genes, the distribution of the low copy fraction of the maize genome, CG content, and other descriptors using linear models and machine learning algorithms.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T10

Enhancement of phosphorus efficiency through genetic improvement of root architecture in maize

(presented by Guohua Mi <miguohua@cau.edu.cn>)
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Phosphorus (P) is one of the essential macronutrients for plant growth and development. A recombinant inbred lines (RILs) developed from two P efficiency contrasting maize of Ye478 (efficient line) and Wu312 (inefficient line) were used to identify quantitative trait loci (QTL) under normal P (NP) and lower P (LP) treatments. Phenotype analysis revealed that P efficiency (PE) showed higher correlation to P utilization efficiency (PUE) than to P acquisition efficiency (PAE) in NP condition, while showed opposite manner in LP condition. QTL analysis revealed 27 QTLs for PE, PAE and PUE from four environments. Four and five QTLs were found to locate at chromosome regions of Bin1.03/1.04 and Bin3.04/3.05, respectively. All alleles from Ye478 in these 9 QTLs presented positive effect to PE, suggesting critical roles of these two regions for maize PE. By maker associated selection (MAS), 31 introgressed lines, which containing at least one chromosome block at Bin1.03/1.04 or Bin3.04/3.05 from Ye478, were selected from 187 BC4F3 lines generated from the same two parents. By comparing to Wu312, these lines showed significant increased grain yield, by average ratios of 12-19% and 22-26% under NP and LP, respectively. One line, L224, showed most yield increase ratio under both NP and LP, was selected for mechanism analysis of introgressed Bin1.03/1.04 and Bin3.04/3.05 from Ye478 into Wu312. By comparing to Wu312, PE, PAE, and root length density in L224 were significant higher in two P conditions, while P acquisition per root length and P transport gene expressions were similar between two lines, suggesting that improvement of PE in L224 might through improving its root length density to explore more space and reach more P. Thus, this work not only put insight in the physiologic and genetic mechanism of PE, but also provided a successful study case of generating P efficient crops.

Funding acknowledgement: Ministry of Science and Technology ‘973’ program (2011CB100305) and ‘863’ program (2012AA100304) of China

T11

Cloning and Characterization of a Major QTL for Northern Leaf Blight Resistance from Two Resistant Lines in Maize

(presented by Bailin Li <Bailin.Li@cgr.dupont.com>)
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Northern leaf blight (NLB), caused by the hemibiotrophic fungal pathogen Setosphaeria turcica (anamorph Exserohilum turcicum), is a major foliar disease in many maize-growing regions, which could result in severe crop loss. The use of resistant hybrids is the most effective and prevalent way to control NLB. Identification and characterization of major resistant QTL to NLB can accelerate the development of resistant hybrids through marker-assisted selection. In this study, two maize inbred lines, PH26N and PH99N, were used in QTL identification for NLB resistance. A major QTL was identified from each line and the QTL co-localized on chromosome 8.06. With the positional cloning approach, NIL-derived mapping populations were used to fine map the QTL and delimit both QTL intervals into the same small physical interval (< 100 kb), suggesting that the QTL are allelic, although the two haplotypes are distinct. BAC libraries from both PH26N and PH99N were constructed, and BAC clones covering the QTL intervals were identified and sequenced. There are two annotated kinase genes within the PH26N QTL interval, while only one kinase gene is in the PH99N interval. Transgenic validation of the candidate genes is underway. Efficacy of the two resistant haplotypes in NLB resistance has been demonstrated in both inbred and hybrid lines in multiple locations in North American and China. The interaction between QTL alleles and locations implies that the haplotypes confer race-specific resistance to NLB. A better understanding of the interaction between the QTL haplotypes and pathogen races will enable more efficient and targeted deployment of the resistant alleles in different breeding programs.

Funding acknowledgement: DuPont Pioneer

T12

A wall-associated kinase ZmWAK confers quantitative resistance to head smut in maize

(presented by Mingliang Xu <mxu@cau.edu.cn>)
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Head smut is a soil-borne systemic disease in maize caused by fungus *Sporisorium reilianum*, and poses a grave threat to maize production worldwide. A major resistance QTL-*qHSR1* has been identified on maize chromosome bin2.09, which could reduce the disease incidence by 25%. Using a recombinant-derived progeny test method, we narrowed down the *qHSR1* region to an interval flanked by markers STS1M3 and STS3M1, spanning 215-kb based on the B73 reference genome sequence. BAC clones covering the *qHSR1* interval in Mo17 (resistant) and HZ4 (susceptible) inbred lines were obtained and subjected to sequencing. There is a 147-kb deletion within the *qHSR1* region of HZ4 genome compared to that in Mo17. Within this 147-kb region, a *ZmWAK* gene encoding a wall-associated kinase is the most likely candidate for *qHSR1*. The transgenic lines with the exogenous *ZmWAK* gene significantly elevated head smut resistance compared to their non-transgenic siblings across multiple generations, demonstrating that *ZmWAK* is the gene underlying *qHSR1*. ZmWAK protein was an active kinase with an extracellular GUB (galacturonan-binding) domain and localized in the plasma membrane. The high expression of *ZmWAK* in mesocotyl at maize early developmental stage inhibited the upward growth of the endophytic *S. reilianum*, and reduced the disease severity. We Investigated PAV polymorphisms of *ZmWAK* in 522 maize accessions and 184 teosinte entries, and found that the *ZmWAK* locus was absent exclusively in maize, but not teosinte, implying the deletion of *ZmWAK* locus occurred after maize domestication and spread among maize germplasm. Introduction of the resistant *ZmWAK* allele by marker-assisted selection would be an efficient way to improve maize resistance to head smut.

Funding acknowledgement: National ‘863’ high-tech program of China

T13

Functional genomics analysis of maize 9-lipoxygenase gene family and 9-oxylipins in the basal resistance to ear rot and stalk rot caused by Fusarium spp. and the induced systemic resistance to anthracnose leaf blight by Colletotrichum graminocola

(presented by Xiquan Gao <xgao@njau.edu.cn>)
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Lipoxygenases (LOXs) catalyze the formation of a large family of poly-unsaturated fatty acid oxide compounds, collectively called oxylipins. While 13-LOXs as well as their ultimate products jasmonates have been well-documented in diverse development and stress-related processes, the function of 9-LOXs and 9-oxylipins have remained to be elusive. It has been hypothesized that plant-derived oxylipins may mimic or interfere with fungal oxylipins to regulate these processes in fungi. To address genetically this question, we investigated systemically the function of 9-LOXs gene family and possible role of 9-oxylipins in the defense response to ear rot and stalk rot caused by *Fusarium* spp. and anthracnose leaf blight by *Colletotrichum graminicola*. Among the 9-LOXs investigated, while we found that *lox3* showed enhanced resistance to ear rot and stalk rot caused by *Fusarium* spp., other 9-LOXs, such as *ZmLOX4* and *ZmLOX5* function as either susceptibility or resistance factors of *Fusarium* pathogenicity. Moreover, disruption of the root-expressed *ZmLOX3* results in dramatic increase in resistance to *C. graminicola* in leaf and shoot, indicating that the roots of *lox3* mutants are the source of increased resistance in leaves. Supporting this hypothesis, treatment of wild-type plants (WT) with xylem sap of *lox3* mutant induced resistance to *C. graminicola* to the levels comparable to those observed in *lox3* mutant. On the contrary, treating *lox3* mutants with the sap collected from WT plants partially restored the susceptibility to *C. graminicola*. Root colonization by *Trichoderma virens* strain GV29-8 induced the same level of disease resistance in WT as the treatment with the mutant sap, but had no additional resistance effect in *lox3* mutant. However, *T. virens* Δsml mutant, which is deficient in ISR induction, was unable to suppress expression of ZmLOX3. Taken together, our data revealed the dynamics and signaling complex of maize 9-LOXs family in defense response against diverse pathogens.

T14

Inference of metabolic pathway dynamics through quantitative genetics: a story of maize grain carotenoids in the nested association mapping panel

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The structural diversity of carotenoids lends to their highly diverse biochemical roles in photosynthesis and germination, signaling, and human dietary nutrition. Existing genetic mapping studies suggest that the primary control on maize grain carotenoid profiles is exhibited by quantitative trait loci (QTL) of metabolic origin from the isoprenoid and carotenoid biosynthetic pathways. Although the effect of single metabolic loci is reasonably predictive of neighboring substrate-product relationships, departure from these predictions has been observed in several breeding studies focused on the combinatorial effect of these pathway genes. These observations suggest that additive gene action insufficiently describes the outcome of the carotenoid profile, and supports the use of a high-resolution genetic analysis to identify causal loci and the nature of their interactions. Leveraging the phenotypic and genotypic diversity of the 5,000 line Nested Association Mapping (NAM) population, we specified a suite of carotenoid synthesis (*PSY1, ZDS, LCYe, CRTRB1, ZEP1, CCD1*) and isoprenoid synthesis (*IPPI, DXS*) genes involved in the regulation of carotenoid concentration. As several of these loci strongly affect multiple carotenoid traits, we tested for pleiotropy within the pathway. Our findings encouraged the use of a multivariate mapping approach to identify regulation at the level of the entire pathway. As a corollary to this analysis, pathway-wide regulation at the level of transcription was assessed through co-expression networks developed from RNA-seq data derived from a catalogue of six stages of kernel development across 22 NAM founder lines. Results reveal that control of the carotenoid pathway is largely regulated at the transcriptional level and offer insights on the non-additive effects of genes within the carotenoid pathway.

Funding acknowledgement: National Science Foundation (NSF)

T15

A GWAS for Trait-Associated SNPs that Exhibit Dominance Effects Provides Insight Into the Origin of Heterotic Groups

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Advances in next generation sequencing (NGS) technologies and the development of appropriate populations and statistical methods have greatly facilitated the genome-wide identification of the genetic determinants of traits. Most commonly this has been accomplished using the frequentist method, which scans the genome one SNP at a time. Using the yield component trait, kernel row number (KRN), phenotypic data from 7,000 inbred and hybrid genotypes genotyped at 13M SNP sites, we compared this classical method of conducting GWAS with a Bayesian-based simultaneous model fitting that has been widely used in livestock species. Many method-specific KRN-associated SNPs were identified and subsequently cross-validated in one or more of three unrelated populations, demonstrating that these two statistical approaches are complementary. Both of these statistical approaches are traditionally conducted using models that assume additive gene action. To better understand the genetic control of heterosis, we analyzed seven yield component traits with varying degrees of heterosis in the 7,000 genotypes using the Bayesian-based simultaneous model fitting approach. The percent of heritability explained by genome wide markers with additive effects was negatively correlated with the degree of heterosis observed for traits. In contrast, the degree of heterosis for a trait was correlated with the number and magnitudes of trait-associated SNPs that exhibited dominant gene action. The trait-associated SNPs that exhibited dominant gene action also exhibited evidence of having been under selection. Finally, the number of such SNPs that would be expected to exhibit complementation via crosses between heterotic groups is significant higher than expected by chance. Hence, this study provides insight into the origins of heterotic groups.

Funding acknowledgement: National Science Foundation (NSF)

T16

Maize ARGOS1 (*ZAR1*) Transgenic Alleles Increase Hybrid Maize Yield

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Crop improvement for yield and drought tolerance is challenging due to the complex genetic nature of these traits and environmental dependencies. This study reports that transgenic overexpression of *Zea mays* ARGOS1 (*ZAR1*) enhanced maize organ growth, grain yield and drought stress tolerance. The *ZAR1* transgene exhibited environmental interactions, with yield increase under Temperate Dry and yield reduction under Temperate Humid or High Latitude environments. Native *ZAR1* allele variation associated with drought stress tolerance. Two founder alleles identified in the mid-maturity germplasm of North America show heterotic group partitioning, with one allele predominant in the female (Stiff Stalk) and the other in the male (Non Stiff Stalk) heterotic groups, respectively. These two alleles are favorable only when heterozygous in hybrids; and have distinct proteins, promoters and expression patterns. Allele-specific transgene testing showed that, each allele had differing impact on yield and environmental interactions. Moreover, when transgenically stacked together the heterozygous allelic pair showed yield performance advantages over either single allele, resembling heterotic effects at this locus. This work demonstrates differences in transgenic efficacy of native *ZAR1* alleles and the differences reflect their association with hybrid maize breeding performance.

T17

Mild drought specifically affects the transition between cell division and cell expansion in the growing maize leaf

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Organ size control is an important trait contributing to plant yield. Cell division and cell expansion are the two main processes driving organ growth. Our lab recently showed both in Arabidopsis and maize leaves that the transition between cell division and cell expansion plays an important role in final organ size determination. Drought is an abiotic stress which causes enormous yield losses, in part by negatively affecting growth. Our group focuses on the molecular networks underpinning yield and organ growth, both under standard and mild drought stress conditions, in Arabidopsis and maize. The mild drought that we apply reduces leaf growth without wilting or leaf rolling. To facilitate the drought research in maize, two growing and phenotyping platforms SHRIMPY and PHENOVISION were built to monitor maize growth in the department of Plant System Biology of VIB. RGB imaging allows both of the platforms to make 3D images providing information on growth rates and biomass accumulation. Kinematic analysis in both B104 and B73 revealed that the growth reduction caused by the mild drought conditions were due to a basal shift in the transition zone, resulting in less dividing cells. To correlate these data to molecular pathways a continuous high resolution sampling throughout the leaf growth zone from the division zone to the elongation zone was subjected to transcriptome analysis. Surprisingly, the majority (57%) of the transcriptomic changes due to mild drought occurred exclusively in the transition zone between cell division and cell expansion, exactly where the phenotypic differences between the two conditions were observed. Therefore, this fine sampling strategy provides a chance to look into the details of molecular and cellular effects of mild drought on growth. This work will shed light on the dynamics of the molecular mechanisms driving leaf growth, both under well-watered and drought conditions and might lead to novel ways to increase yield stability.

T18

Maize Genotyping using RAPiD-Seq (Randomly-Amplified Polymorphic DNA Sequencing)

(presented by Leandro G. Neves <jmcguire@rapid-genomics.com>)
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Genotyping based on sequencing (GBS) has become standard for maize genomic analysis. GBS approaches typically use restriction digestions of DNA, ligation of adaptors and PCR amplification, followed by next-generation sequencing and alignment. GBS is limited to the portion of the genome sampled by existing types of restriction enzymes and involves several enzymatic steps. Also, large numbers of low-depth reads are observed, requiring extensive data imputation and increasing error rate. We assessed an alternative to GBS – Randomly-Amplified Polymorphic DNA sequencing (RAPiD-Seq), which is based on a single PCR reaction using primers containing a combination of degenerate and specific bases. RAPiD-Seq needs less than 100 ng of DNA, and modifications to the specific sequences in the primer permit evaluation of a nearly unlimited number and distribution of genomic regions. To evaluate RAPiD-seq in maize, we genotyped the reference genome B73, 25 parents from the NAM population, 70 NILs derived from those parents plus 12 randomly repeated samples. A total of 59,554 SNPs were identified in the population with median sequencing depth of 31× and a reproducibility of genotypic call of 0.99 when 12 random samples were independently repeated. Markers were equally distributed with a median interval of 56Kbp. A panel with 5K and 20K markers were selected and consistently genotyped with an estimated multiplexing of 144 and 96 samples per HiSeq lane. NIL samples had a median heterozygosis of 0.0047 and genotypic data was used to identify introgression blocks. Results suggest that RAPiD-Seq is a viable and flexible method for maize genotyping.

Funding acknowledgement: RAPiD Genomics LLC

T19

Gramene: A Resource for Comparative Plant Genomics

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Gramene (http://www.gramene.org) is a curated resource for comparative functional genomics in crops and model plant species, with components produced in collaboration with the plants division of Ensembl Genomes. Its strength derives from the application of phylogenetics and integration of genome annotation and functional data using ontologies. The current release features 28 complete reference genomes for monocots, dicots, and lower plants, including the updated maize B73 assembly and gene build RefGen_v3, and an assembly converter tool to navigate between this and RefGen_v2. Recently added species include tomato, potato, barley, banana, foxtail millet, Medicago, Chinese cabbage, wheat and wheat progenitors, wild and domesticated rice and rice progenitors. For each reference genome, we incorporate community annotation from primary sources followed by enriched functional annotation by InterProScan and classification using controlled vocabularies, Gene/Plant Ontology (GO/PO). Evolutionary histories are provided by Compara phylogenetic gene trees and complemented by analyses of whole-genome alignments. In recent years Gramene has positioned itself as a resource for genome variation data. The current release includes maize HapMap2, a collection of 55 million SNPs and InDels from over 100 germplasm accessions, and an upcoming release will include two new large collections of maize variants. In addition, Gramene hosts variants in rice (*O. sativa* and *O. glaberrima*), Arabidopsis, barley, sorghum, wheat, grape, and Brachypodium.

Gramene also produces and hosts metabolic pathways databases and visualization tools. This release includes the latest update to MaizeCyc. In addition, we recently released the Plant Reactome (http://plantreactome.gramene.org), a platform for the comparative analysis of plant metabolic and regulatory networks, featuring at present curated rice pathways. Forthcoming, Plant Reactome will include Arabidopsis pathways and orthologous projections in maize. Gramene is supported by an NSF grant (IOS-1127112) and works closely with the EBI-EMBL, the OICR, and the ASPB.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T20

Mutagenesis; Characterization and Evaluation

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Maize is especially useful for the study of genetic phenomena found in all other species. We have investigated most of the known options for inducing mutations in maize and have found the chemical reagent, EMS was highly mutagenic when used with a paraffin oil carrier on corn pollen. We produced thousands of mutants of which 3484 are characterized. We present a summary characterization and evaluation of the active agents.

1. Spontaneous mutants already available and those rare new ones that were easily recognized in our untreated control populations but not individually identifiable as such in the treated populations.
2. Cytogenetic manipulation, chromosome breakage, ploidy and naturally occurring instability.
3. Radiation: UV light, X-ray and Atomic ionizing radiation.
4. Transposons; Naturally occurring systems which show genetic instability but few adverse effects.
5. Chemical reagents that interacted with DNA; were destructive to living cells and therefore difficult to use.

We investigated all and found only 4 and 5 were promising. We discovered a technique by which biologically disruptive chemicals like EMS could be applied to fragile germ cells (corn pollen) producing a dramatic increase in all kinds of heritable changes. We had more mutants than existed anywhere else in the world. We decided to share them with our colleagues, to characterize as many as possible, and create a data base with pictures of the many phenotypes seen.

The results appear in other presentations at this conference; the Mutant Image Catalog at MaizeGDB, mutant stocks at the Maize Genetics Stock Center, the book “Mutants of Maize” and especially a web Wiki entitled “Guide to Maize Mutant Phenotypes”.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T21

Characterization of Maize Autophagy System Reveals a Central Role in Nitrogen Remobilization

(presented by Faqiang Li <fli32@wisc.edu>)
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Plants, like other organisms, employ autophagy as a major route to recycle intracellular constituents during cell or tissue remodeling, to clear damaged organelles and toxic cellular debris that accumulate, and to better survive when nutrients are limiting. This process involves the sequestration of cytoplasmic material into autophagic vesicles and subsequent delivery of the vesicles and cargo to the vacuole for breakdown. Central to autophagy is the conjugation of the ubiquitin-like protein AUTOPHAGY-RELATED (ATG)-8 to the lipid phosphatidylethanolamine (PE) by an ATP-dependent enzymatic cascade sequentially involving ATG7, ATG3, and the ATG12-ATG5-ATG16 complex. The ATG8-PE adduct then decorates enveloping autophagic vesicles to promote their enclose, as well as serving as a docking site for factors that recruit appropriate cargo. To help define the importance of autophagy to agriculture and its impacts on crop yield, we have begun characterizing the maize Atg system at the genetic and biochemical levels. From searches of the various transposon insertion populations, we have identified potentially useful mutations affecting several *ZmAtg* loci, including two for *ZmAtg12*. Both *Zmatg12* alleles accumulate longer, *Mu*-containing transcripts that eliminate the C-terminal amino acids essential to ZmAtg8 lipidation. Homozygous *atg12* plants fail to generate the ZmAtg8-PE adduct and accumulate YFP-Atg8a-labeled autophagic vesicles inside the vacuole. Phenotypic analyses showed that the *atg12* plants are fertile and relatively normal phenotypically when grown on nitrogen-rich soil. However, when grown under nitrogen limiting conditions, young seedlings display more severely retarded root and shoot growth, and as the plants mature, they show enhanced leaf senescence and delayed ear development as compared to wild type. Together, our studies demonstrate that Atg8-mediated autophagy is not essential to maize, but becomes critical to growth and development during nitrogen starvation by helping promote recycling. As such, the Atg system might be a key determinant of crop productivity under suboptimal field conditions.

Funding acknowledgement: National Science Foundation (NSF)

T22

Expression of *Arabidopsis thaliana* HB17 gene in corn leads to improved sink potential

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As a result of the large scale screening of candidate genes in transgenic corn, we identified *Arabidopsis thaliana* HB17, a member of homeodomain-leucine zipper II (HD-Zip II) family of the plant transcriptional factors, which affects plant growth and leads to increased ear size at silking. When expressed in corn, AtHB17 lacks the repression domain due to the corn-specific splicing mechanism and loses the ability to bind the co-repressors and affect transcription of the target genes. The protein still can form homo-dimers as well as hetero-dimers with corn endogenous HD-Zip II proteins and bind to the target DNA sequences due to the presence of the functional leucine-zipper and DNA-binding domains. We propose that AtHB17 expressed in corn mediates physiological effects through dominant-negative mechanism by attenuating transcriptional repression activity of endogenous corn HD-Zip II proteins. We hypothesize that modulation of the activity of HD-ZIP II proteins leads to modulation of corn plant's growth responses to environmental and developmental signals and, ultimately, to increased ear size, thus, providing opportunity for enhanced sink potential in corn plants. Increased sink potential could be manifested through an increase in kernel weight or kernel number depending on the environmental conditions.

T23

ZmHDA108 has an active role both in setting the histone code and controlling plant vegetative and reproductive development of maize.

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Hypoacetylation of histones mediated by histone deacetylases (HDAs) is associated with a more compact chromatin structure, which blocks the accessibility of transcription factors to target sequences and leads to the repression/silencing of genes. Maize ZmHDA108 belong to the Rpd3/HAD family of plant histone deacetylases and the corresponding genes are expressed ubiquitously during maize development. An insertional lines for the *ZmHDA108* gene was identified by BIOGEMMA within their Mutator transposon insertional maize mutant collection. Insertional mutants were back-crossed five times to B73 and then two selfing cycles were performed.

The phenotype of *hda108/hda108* mutant plants indicate that the *HDA108* gene knock-out is correlated with many developmental defects. Compared to wild-type plants, homozygous plants present a significant reduction of plant height, alterations of shoot and leaf development (shoots curvatures, leaf blade reduction, leaf twisting, leaf knots, disorganized differentiation of the blade-sheath boundary). During reproductive development, they present alterations in female inflorescence patterning: unusual ear axis elongation is correlated with an abnormal ear differentiation and reduction of fertility. Similarly male fertility is corrupted: abnormalities affect both tassel development and pollen differentiation. Furthermore, the double mutant *hda101hda101/hda108hda108* kernels have both defective embryo and endosperm resulting in a lethal phenotype.

Immunolocalization experiments reveal an alteration of epigenetic mark distribution in *hda108/hda108* root tip nuclei isolated from BC5S1 seedlings. Different histone modifications (e.g., H3ac, H3K9ac, H3K9me2) were analyzed using specific antibodies. Confocal microscope observations of immunolabeled nuclei reveal an evident increase in histone acetylation in homozygous mutant nuclei compared with wild-types while H3K9me2 shows marked reduction in mutants. In mutants the expression of *HDA genes* of the RPd3/HDA family is altered in different tissues and in defective tissues the expression of *KNOX* transcription factors (putative HDA108 targets) is deregulated.

T24

Impairment of a Chitinase-like1 is responsible for the phenotype of a brittle stalk mutant of maize

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The maize (Zea mays) brittle stalk4 (bk4) mutant was identified in a Mutator F2 population. All aerial parts of the plant such as the leaves, stalk, midrib, brace roots, and tassel were highly brittle and easily breakable in mechanical or high wind conditions. Mutation of bk4 causes reduction in average stalk diameter and mechanical strength. In addition, the mutant has a dwarf stature, senescence at leaf tip and semi-pollen sterility. The mutant phenotype is due to a single recessive gene mutation, mapped to chr7L, and non-allelic to both brittle stalk2 (bk2) and bk3, thus, designated as bk4. Histology and biochemical studies detected significant differences not only in shape and size of vascular bundles of leaf midrib and stalk pith of bk4 mutant but also in lignin staining and reduction in p-Coumaric acid, glucose, mannose, and cellulose contents as compared to its wild-type sibs. A candidate gene responsible for bk4 phenotype was isolated by co-segregation analysis and validated by studying additional two independent mutant alleles and their expression analysis. bk4 encodes for Chitinase-like protein1 and is expressing highest in elongated internodes. Expression levels of secondary cell wall cellulose synthase genes (cesA) in single bk4 mutant; and phenotypic observations in double mutants of bk4 in combination with bk2 or null alleles for secondary cell wall cesA, indicate that bk2 is downstream of bk4 and secondary cell wall cesA genes are acting upstream of ZmCtl1. Over expression of ZmCtl1 in FAST corn plants resulted in enhanced mechanical stalk strength without epistatic effects of other plant traits which were effected in knockout bk4 mutations. Further biochemical characterization of transgenes will be discussed.

T25

Distinct small RNA populations act antagonistically in heterosis formation of maize

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Heterosis, a well known phenomenon describing the superior performance of hybrid offspring over their parents, with high relevance not only to adaptive evolution but also great value to man in dramatically increasing yield by hybrid breeding. Small, non-coding RNAs (sRNAs) are known to be responsible for regulation of gene expression and modulating the epigenomic landscape in diverse organisms. In plant hybrids sRNAs undergo tremendous expression changes and are attributed to play fundamental roles for the specification of hybrid phenotypes. The mobility of sRNAs makes them ideal candidates for key regulation processes in the interplay of diverse genomes in trans. We revealed that sRNA populations of maize parental inbred lines hold high variability and found significant associations between distinct sRNAs of a large set of maize inbred lines and heterosis of their hybrid offspring. The majority of the sRNAs identified exhibit a negative association with heterosis and in agreement, reducing sRNA complexity in transgenic plants significantly increases the heterotic outcome in hybrids. Together, we showed that sRNAs contribute to heterosis formation and may be major players in shaping the phenotypic outcome of newly synthesized hybrids. The uncovered restraining effects of sRNAs on heterosis suggest that future plant breeding strategies that include epigenomic information are able to generate unprecedented levels of heterosis and considerably increase crops yield by using epigenomic information.

Funding acknowledgement: DFG

T26

De novo assembly of Maize Mo17 genome and its comparison with B73 genome

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Zea mays, domesticated from the wild grass teosinte about 9,000 years ago, remains a relatively diverse species despite extensive improvement. However, most genomic research in this crop is based on the reference genome of one inbred, B73. To further assess maize genome complexity, including structural variation, fully assembled genomes of other important maize lines are needed. Here we report the De novo assembly of a classic inbred Mo17. We obtained 115-fold depth of short reads from libraries with various insert sizes and assembled 2.24-Gb scaffold sequences (2.04-Gb nongapped sequences) with an N50 length of 26-kb. About 80% of the Mo17 assembly was identified as repetitive elements. We used an evidence-based approach to annotate about 42,000 genes in Mo17 genome. A LD-mapping approach was used to cluster scaffolds into ~3,000 clusters and anchored to the chromosomes, which 70% of the genome (1.5-Gb) and 85% of genes. A whole genome comparison with B73 genome identified nine million SNPs, one million INDELs, and PAV for about two thousand genes. Details of the Mo17 assembly and its comparison with B73 genome will be presented at the meeting.

Funding acknowledgement: 973 program, 863 project

T27

Genomic, transcriptomic and phenomic variation reveals the complex adaptation of modern maize breeding

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The temperate-tropical division of early maize to different agricultural environments was the greatest adaptation process associated with the creation of a stable food supply, which in turn allowed the development of human civilization not only in the Americas but the whole world. Deciphering this history is always challenged, however, genomic variation, transcriptomic changes and phenotypic data were collected from 368 diverse temperate and tropical maize inbred lines in this study to first systematically explore the mechanisms of the adaptation process.

Results indicate that divergence between tropical and temperate lines occurred 3,400-6,700 years BP. A number of genomic selection signals and transcriptomic variations including differentially expressed individual genes and rewiring co-expression networks were identified. These candidate signals were found to be functional related to stress response and most were associated with the directional selected traits, which maybe advantage in phenotypic performance to widely varying environmental conditions faced by maize as it was migrated far north and south of its domestication center. We believe these joint results should increase our knowledge and resources for developing breeding strategies to cope with the increasingly erratic climate.

Such stress adaptation, involving protein-coding sequence evolutionary as well as transcriptome-level regulatory changes were observed in this study. However, transcriptome regulation could be more flexible and dynamic and may allow maize to adapt to environmental changes over this dramatically short evolutionary time frame.

Funding acknowledgement: National Hi-Tech Research and Development Program of China, National Natural Science Foundation of China, Fundamental Research Funds for the Central Universities

T28

Machine vision phenotyping uncovers novel relationships between kernel and seedling characteristics.

(presented by Jeff Gustin <jgustin@ufl.edu>)
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Phenotypes are currently not studied with the same degree of sophistication or throughput as genomes. Because phenotypes are such an important source of information about gene function, we are integrating multiple machine vision platforms to study seed and seedling phenotypes. We have developed a semi-automated pipeline to collect kernel weight, near infrared reflectance (NIR) kernel spectra, kernel color and 3D shape, and dynamic seedling root growth. Kernels from 27 Nested Association Mapping (NAM) inbred lines in addition to W22 were serially phenotyped using these machine vision platforms. To account for potential confounding ear position effects, kernel coordinates were recorded and used to generate ear maps for each of the NAM parent kernel populations. Complete longitudinal data were collected for over 1200 kernels in this dataset. A combination of principal components analysis (PCA) and canonical correlations analysis (CCA) were used to establish overall patterns between datasets and to identify subsets of variables with potentially significant relationships. CCA indicated that kernel composition, as estimated by NIR, and kernel size were significantly correlated with multiple aspects of root growth dynamics. Kernel weight was significantly correlated with growth rate response after gravitropic stimulation. Lighter seeds showed greater acceleration, higher maximum speed, and faster deceleration than heavier seeds. The rate of gravitropic response was negatively correlated with kernel volume and positively correlated with percent of oil in the seed. These data suggest smaller seeds respond faster to environmental queues. In general, genotype has a significant effect on many of the observed relationship with some genotypes showing contrasting correlations to the general model. This work suggests kernel phenotypes can be used to select kernels with greater potential to improve agronomic performance.

Funding acknowledgement: National Science Foundation (NSF)

T29

Gene and genome changes in the grasses

(presented by Jeff Bennetzen <maize@uga.edu>)
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Comparative genomic analysis of the grasses has been a particularly powerful tool for maize evolutionary and functional genetics over the last twenty-five years. Our recent studies include analyses of centromere, gene pair, indel, intron and transposable element dynamics. Using a few examples from these studies, we will present the major molecular mechanisms responsible for grass genome instability, and discuss the driving forces behind these changes.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

Poster Abstracts

P1
A Lightened Path for Dissecting Maize Flowering Time by Using Genome-Wide Association Study

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Maize flowering time is complex trait that controls local environment adaptation and has been intensively selected in corn breeding. The linkage analyses revealed that at least forty genes, each with a small effect, were involved for flowering time. This provoked Genome-Wide Association Studies (GWAS) due to lacking of statistical power, especially on small samples. For example, there was barely association signal for flowering time from the 282-association panel when the 55,000 SNPs from the Illumina array were tested one at a time. We developed an algorithm to consider multiple SNPs simultaneously in a fixed model and use a random model to perform the optimization. This algorithm of FARM-CPU (Fixed And Random Model Circuitous Probability Unification) had strikingly high statistical power. In the example of 282-association panel, multiple loci were identified, including the associations on known genes reported previously. The algorithm was also computationally efficient. The association analysis can be accomplished within an hour for the Ames collection with more than two thousands individuals genotyped with half millions SNPs. This new algorithm would increase the probability of success to dissect genetic architecture of complex traits by using GWAS.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P2
Analysis of the repeat sequences in *Coix lachryma-jobi* and *Coix aquatic* genomes

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Coix,an oriental genus of the tribe *Maydea*,is the closest group to the genus *Zea* after *Tripsacum*. This genus contains ten species at different ploidy levels, such as diploids (2n = 10), tetraploids (2n = 20) and hexaploids (2n =30) . Six GB genomic sequences of *Coix lachryma-jobi* (2n=20) and nine GB genomic sequences of *Coix aquatic* (2n=30) were used for analyzing repeat elements. The graph-based methods for similarity-based clustering of reads were used to reveal the genome structure of these two species, and we found that approximately 76% of the *Coix lachryma-jobi* genome and 73% of the *Coix aquatic* genome consist of repeat sequences. The proportion of LTR retrotransposon, a dominant repeat element, is 69% in *Coix lachryma-jobi* genome and 64% in *Coix aquatic* genome. Comparison of the genome structure exhibited large proportional change of the same element between two genomes, indicating evolutionary divergence of these two species. We further analysed seven most dominant satellites and found five common satellites with large proportional change between these two genomes, and the other two satellites only detected in *Coix aquatic*. All these satellites were used for the karyotype analysis. Our data suggested genome restructuring in these two close relatives after their speciation. Besides, the genome comparison of *Zea Mays*, *Zea luxurians*, *Sorghum bicolor* and *Coix* indicated that repeat sequences diverged after the speciation of these species from the same ancestor. Our results shed new light on the evolution and species differentiation in the *Gramineae*.

P3
Biology of Rare Alleles in Maize and Its Wild Relatives

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The biology of rare alleles is fundamental to our understanding of evolution and genotype-to-phenotype relationships. However, it has yet to be adequately explored in any system. This project is combining the power of population genetic and molecular models with quantitative genetics to elucidate the relative contributions of rare versus common alleles to phenotypic variation and evolution. We are taking advantage of recent advances in high-throughput genotyping and phenotyping methodologies to identify the key biological attributes of variants ("polymorphism descriptors") that will allow us to better predict the functional effects of rare alleles in Zea. This project will refine our understanding of natural phenotypic variation, which is critical to genetics, medicine, agriculture, and conservation. On a practical level, this research is providing tools to identify deleterious SNPs in maize individuals, and to estimate their overall number and distribution in populations. This information can then be used in genomic selection or future homologous recombination approaches for crop improvement. This will facilitate the use of diverse genetic resources such as landraces, or even teosinte, in elite breeding programs. The effectiveness of plant breeding will be enhanced by improving our ability to identify, predict, and select on the effects of rare variants.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P4

Breeding maize for resistance to Fusarium ear rot: a candidate gene approach from the integration of metabolomics and transcriptomics.

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Fusarium verticillioides is the causal agent of Fusarium ear rot in maize and contaminates the grain with fumonisins, a family of mycotoxins that affects feed and food. Candidate genes for kernel resistance to F. verticillioides infection were detected through the comparison of resistant (CO441) and susceptible (CO354) maize inbred lines, through transcriptomic (RNASeq) and metabolomic analyses. We observed 2,250 and 2,442 differentially expressed genes at 72 hours post inoculation (hpi) for the resistant and susceptible maize genotypes, respectively, of which 1,028 were in common and showed 5,342 SNP variants. Our data indicated that the resistance in CO441 was based on differences in basal gene expression between the two genotypes. At 72 hpi the transcriptional modulation remained higher in the resistant line, involving the specific changes of numerous transcripts encoding components of signal transduction cascades and enzymes required for the synthesis of secondary metabolites.
The metabolic profiles of the same kernel samples were investigated with Liquid Chromatography-High Resolution Mass Spectrometry. Stable isotopic labeling combined to MetExtract algorithm allowed the automatic detection and prediction of carbon atoms of biological metabolites. 830 and 1135 peaks resulted in negative and positive ionization mode, respectively. Metabolite annotation was achieved by searching accurate m/z values, carbon atom numbers and type of ion species in MaizeCyc and KEGG databases. Statistics on consistently detected metabolites (around 85%) demonstrated a clear separation between maize genotypes and treatments. A large amount of metabolites and transcripts resulted involved in defense pathways: biosynthesis of aromatic amino acids, phenylpropanoids, flavonoids and oxylipin metabolism.
The identification of differentially expressed plant genes and metabolites after pathogen interaction will produce useful tools for the identification of candidate genes, the development of molecular markers and their use for selection of resistant maize genotypes by means of marker assisted selection.

P5

Comparative Proteomic Analysis of Salt Stress Responses in the Roots of Two Maize (*Zea Mays* L.) Inbred Lines at the Early Seedling Stage

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Salinity is a major abiotic stress limiting plant productivity and quality all over the world. Roots are the sites of salt uptake and respond immediately to salt stress. A comparative proteomic analysis of seedling roots was performed using an iTRAQ approach from the salt-tolerant genotype F63 and the salt-sensitive genotype F35 that were exposed to 160 mM NaCl for 2 days. Under high salinity, fresh weight and relative water content were significantly higher in F63 than those in F35, on the contrary, osmotic potential and the reduction of K+/Na+ ratio were significantly lower in F63 than those in F35. Ninety-three and forty-nine proteins of salt-treated samples showed more than 1.5 fold changes in abundance in F63 and F35, respectively. The proteins exhibiting the similar induced profiles indicated that the salt-tolerant and salt-sensitive genotypes had the common salt respond mechanisms under salt stress and these proteins were involved in the process of hormone regulation, stress/defense, and water conservation. However, the proteins exhibiting the different induced profiles may contribute to the difference of the two maize genotypes in salt tolerance and these proteins were mainly involved in oxidative stress and protein synthesis.

Funding acknowledgement: China National Science Foundation (Grant No. 31201214), National Sci-Tech Support program (Grant No.2013BAD05B01)

P6

Comparative transcriptomic analysis of primary, seminal and crown roots of maize

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The maize root system is essential for plant development, anchorage and acquisition of water and nutrients, and is therefore of great potential for future crop productivity improvement. The maize primary and seminal roots form the embryonic root system which dominates maize seedling development, while crown roots are formed post-embryonically and make up the major portion of the adult root stock. Although the various embryonic and post-embryonic root-types of maize have a similar morphological structure, they are initiated at different stages of development from different tissues. Characterization of root-mutants revealed genes that are involved in the regulation of specific root types. Histological analyses of transverse sections of the three root types demonstrated that seminal roots display a smaller diameter resulting from a reduced number of cortical cell layers and a smaller stele area compared to the other root-types. Moreover, crown roots display a larger number of metaxylem elements than primary and seminal roots. Based on the morphological and anatomical similarities and differences of the three root-types we will explore conserved and distinct mechanisms associated with the development of the three root types. To identify differentially expressed genes involved in the formation of the three different root types, 2-3 cm long roots of the three root types will be subjected to RNA-seq experiments to obtain novel insights into the complex molecular networks involved in the development of maize embryonic and post-embryonic roots.

P7

Comparing Rates of Changing Repetitive Content to Rates of Genic Nucleotide Polymorphism in Maize Landraces and Their Relatives in *Zea* and *Tripsacum*

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Structural variation plays an important role in generating diversity in the maize genome and is known to contribute to fluctuations in genome size. Using next-gen sequence data, we investigate changes in repetitive content in populations of maize landraces and teosinte across an altitudinal gradient in Mexico to identify the repetitive content responsible for genome size variation. We focus on transposable elements (TEs), heterochromatic knobs, and tandem centromeric repeats because together they can account for a vast majority of the genome. After correcting for genome size variation in these populations, we compared abundance of these three repetitive classes in a diverse panel of 500 different maize landrace, teosinte, and gammagrass individuals. We find a number of environmental trends, the strongest being a correlation between altitude and genome size. Surprisingly, different classes of repeats showed opposing clinal patterns, suggesting that both simple neutral models and models of selection against genome size are insufficient to explain the data. Treating genomic content as a phenotype, we further compare rates of repetitive content flux to SNP variation between populations to reveal repetitive content can also very strongly differentiate populations.

Funding acknowledgement: National Science Foundation (NSF), UC MEXUS

P8

Deep analysis on maize genome sequencing data reveals that the presence/absence variation (PAV) was comprehensively involved in disease resistance

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Understanding the genome structure variation that contributes to phenotypic diversity would facilitate the exploration of agricultural traits with great importance in maize. Presence/absence variation (PAV), a major class of genome structure variations, is believed to be pervasive in the whole genome. The studies with array comparative genomic hybridization (CGH), have reported the presence/absence variation (PAV) in B73. However, due to technical limitations, only PAVs that are present in the B73 reference genome but absent from the Mo17 genome were located. Here, using the next generation sequencing, we investigated the PAVs that are present in Mo17 but missing from B73 by using a series of bioinformatics pipelines. 119 PAVs were in silico detected, and half of them were PCR validated. With IBM (intermated B73 x Mo17) segregating population, we genetically mapped 39 PAVs on the linkage map, and found that PAVs dispersed in the chromosome unevenly. The annotation of the PAVs revealed that several PAVs contained some disease resistance genes, such as stem rust resistance genes. The expression of gene harbored in PAVs was almost fully silenced or expressed at a relative low level using RNA-seq data of Mo17. The PAVs characterized here offered compensation to the previous studies and provided the insight to understand the function of PAVs.

Funding acknowledgement: National Natural Science Foundation of China (NNSFC)

P9

Dissect the Genetic Architecture of Nitrogen Response in Maize

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In the past decades, nitrogen fertilizers played a key role in increasing crop yields, however, the nitrogen use efficiency (NUE) of crops has actually decreased due to the overuse of nitrogen. Despite large numbers of genetic studies on NUE have been conducted, the genetic regulatory mechanism of nitrogen response is still not well understood owing to its complexity. With the rapid developments in next-generation sequencing technologies, RNA-seq becomes a powerful tool for dissecting complex traits. In this study, we combine RNA-seq and genetic mapping to dissect the genetic architecture of nitrogen response in maize. By differential expression analysis on RNA-seq data of leaf tissue at days to anthesis for Zheng58 and Chang7-2 (parents of Zhengdan958, a hybrid with the largest planting area in China) under two field nitrogen conditions, the expression of 95 genes were shown with significantly different nitrogen response pattern between Zheng58 and Chang7-2, suggesting that Zheng58 and Chang7-2 contain genetic differences at these genes that drive to produce different nitrogen response pattern. Of 95 differential expressed genes, 85 genes were more sensitive to nitrogen in Chang7-2 than in Zheng58. The 95 differentially expressed genes were significantly enriched in transcription factor and ligase activity, and further GO term results showed that they were significantly enriched in the class of AP2/ERF and WRKY transcription factors. Simultaneously, using a recombinant inbred line (RIL) population derived from the cross between Zheng58 and Chang7-2, genetic QTL mapping for nitrogen response difference between two field nitrogen conditions for seven important agronomic traits was conducted. A total of 30 QTLs were detected for nitrogen response related traits. Very interestingly, differentially expressed genes identified by RNA-seq appeared to be significantly enriched in the supporting intervals of QTLs for nitrogen response detected in RILs. This result suggests that differentially expressed genes between parents can be stably inherited into progeny and can explain a substantial proportion of phenotypic variation. This study provides a good example to demonstrate that combining RNA-seq and genetic mapping might be a powerful strategy to dissect complex traits. The further characterization of differential genes identified in this study could further advance our understanding of the genetic regulatory mechanism of nitrogen response in maize.

Funding acknowledgement: National Natural Science Foundation of China (NSFC), National High-tech Research and Development Projects (863)

P10
Evaluating Evidence for Centromere Drive in the Family Poaceae

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Centromere drive has been hypothesized to occur when chromosomes with long centromere repeat arrays more extensively recruit kinetochore proteins and spindle fibers relative to chromosomes with shorter arrays. Chromosomes with longer arrays may migrate more quickly along the spindle apparatus during meiosis and thus be preferentially incorporated into the female gamete. This process would have profound evolutionary implications, potentially leading to rapid differentiation and speciation. Drive could be counteracted, however, by evolution in the DNA-binding domains of kinetochore proteins. We have resequenced DNA-binding domains of the foundation kinetochore proteins CenH3 and CenPC in samples of maize, its wild relatives, and several species from the broader Poaceae. In addition, we have generated full-genome, short-read data from these individuals in order to estimate centromere repeat abundance. We report here new analyses of sequence from DNA-binding domains through which we evaluate evidence for episodic selection. We also treat our estimates of centromere repeat abundance as phenotypes for each species and test the utility of Brownian Motion and Ornstein-Uhlenbeck models for describing evolution of this trait across the phylogeny. Collectively these analyses provide insight into the potential evolutionary role of centromere drive in Poaceae.

Funding acknowledgement: National Science Foundation (NSF)

P11
Exploring Gene Regulatory Network of miRNAs in Maize

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MicroRNAs (miRNAs) are important non-coding RNAs that play a central role in plant development and in the response to environmental stress. We are interested in studying the Gene Regulatory Network (GRN) that regulates miRNA expression in the plant. Most miRNAs are transcribed by RNA polymerase II and their transcription process is regulated by transcription factors (TFs). In order to systematically resolve points of crosstalk among TFs, miRNAs, and their targets in a comprehensive way, we make use of a gene-centered yeast one-hybrid (Y1H) approach. We have developed a TF library that represent 95% of TFs expressed in Arabidopsis root, 150 miRNA promoters and their targets had been screened using this library and obtained close to 4000 Protein-DNA interactions (PDIs), representing 2.3% of possible protein-DNA interactions. This network had been projected to maize with same miRNA families and TFs orthologs found in the Arabidopsis GRN using this network topology information and available RNA-seq/microarrays data from public dataset. Subsets of the projected maize network were validated by screening their promoters using both Arabidopsis TF library and their corresponding maize ortholog library. Here, we are reporting the conservation levels of miRNA-based GRNs between Arabidopsis and maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P12

Extensive regulatory neofunctionalisation following whole genome duplication in maize

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Whole genome duplications are a dominant feature of plant genome evolution, and have been detected in all angiosperm lineages. Maize underwent a whole genome duplication event between 5 and 12 million years ago. In contrast, the sorghum genome has retained the ancestral non-duplicated state. Post-duplication, some duplicated genes are retained in the genome as homeolog gene-pairs. If retained, the individual genes in a homeolog gene-pair may have identical functions, alternatively they may partition and share the original gene function (subfunctionalisation), or they may diverge and develop novel functions (neofunctionalisation). Neofunctionalisation of duplicated homeolog gene-pairs has long been proposed as a major source of evolutionary innovation, however, despite constituting a large fraction of the maize genome, it is not yet known to what extent homeolog gene-pairs have diverged in function since the whole genome duplication event. We have sought to assess functional divergence using transcriptomics in the context of foliar and husk leaf development, and bundle sheath and mesophyll cell gene expression. Through this we find extensive evidence that homeolog gene-pairs have diverged in expression profiles in maize leaves. These divergent gene-pairs are frequently under higher purifying selection, and exhibit greater levels of subgenome expression dominance early in leaf development. We also find that specific types of genes are more likely to diverge in expression. Our findings have important implications for our understanding of genome evolution, leaf development and photosynthesis.

Funding acknowledgement: Newton Abraham Scholarship, BBSRC, Leverhulme Trust, Bill and Melinda Gates Foundation

P13

From seed to senescence: Transcriptome tools to understand maize development, physiology, and phenotypic diversity

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The transcriptome provides insights into biological and developmental processes that occur throughout the life cycle of a maize plant. In this presentation, we will describe biological interpretations and technical strengths and limitations of two large sets of RNA-seq data that will be released to the maize community. We have created an expanded RNA-seq based B73 gene atlas encompassing 81 RNA samples analyzed in triplicate. These include 52 of the original array-based gene atlas samples, a time-course of 12 stalk and leaf samples post-flowering, and a new set of 17 samples from the maize seedling and adult root system. Examples of enhanced resolution of gene family members, increased utility of network analysis, and value of the data set in identifying promoter motifs will be described. We have also developed RNA-seq data from seedlings of 670 diverse inbred lines and a small set of doubled haploids. This data set has proven to be a valuable resource for identification of non-reference genes, SNP detection, and genome-wide association analysis. We will describe use of expression level to detect GWAS associations, interpretations from eQTL analysis, and relationships between the gene atlas and diversity transcriptome in assessing gene networks and gene expression. The RNA-seq datasets that we have generated have proven extremely powerful in understanding maize development, physiology, and phenotypic diversity and are expected to be valuable tools to the maize community.

Funding acknowledgement: Department of Energy (DOE)

P14
Functional annotation of B73 gene models: A machine learning approach

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Functional annotation of genes is a crucial step to derive useful information from the genome assembly of any organism. The **G**ene **O**ntology (**GO**) is a structured set of hierarchically related terms that describe molecular functions, biological processes, and cellular localization. Historically, **GO** term assignment to gene models have been based on a simple method whereby terms are simply inherited based on sequence similarity to a previously annotated genome. However, when only sequence similarity is used, an incorrect assignment in the original species is inherited by other species and errant functional annotations are propagated. Machine Learning approaches can be used to overcome this limitation by expanding the input from simple sequence similarity to a broad range of more functionally relevant sequence-based inputs and by assessing previously assigned annotations from a group of genes across various species prior to term assignment. We have selected several sequence based features (e.g., amino acid composition), and domain based features (Pfam) for testing and are currently evaluating different machine learning algorithms on select test cases. Here we describe our pipeline to create high-confidence **GO** associations for maize gene models based on a supervised Machine Learning approach and show how the method performs relative to simple sequence similarity-based approaches.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P15
G-quadruplex motifs are found in genes regulated by hypoxia, low sugar, and nutrient deprivation in maize (*Zea mays ssp. mays L.*)

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The 4-stranded G-quadruplex (G4Q) elements are abundant, cis-acting elements in DNA and RNA that function in maintenance and expression of genes in prokaryotes and eukaryotes. To investigate their roles in the plant kingdom, we computationally identified potential G4Qs in the maize (*Zea mays L.*) genome. We found 149,988 non-telomeric G4Q motifs, with 43,174 remaining after repeat sequence masking. Nearly one quarter of the filtered gene-set transcripts contained one or more G4Q motifs, with positional hot spots occurring in the antisense/template strands of the 5' UTR and of the 5' end of the first intron. Representative genic G4Q oligonucleotide sequences showed quadruplex formation *in vitro*. G4Q-containing genes were over-represented in metabolic pathways related to hypoxia, glycolysis, sugar degradation, inositol metabolism, and base-excision repair. In addition, G4Qs were prevalent in genes for signaling pathways, including the hypoxia response, AMPK/SnRK, and DJ-1/GATase1. From these results, we propose that maize G4Q are ideally positioned to aid expression of genes involved in adaptive metabolism of low-oxygen or low-sugar conditions. The G4Qs are therefore likely to have widespread and previously unrecognized significance in linking energy crisis perception to genomic response in plants.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P16

Metabolic Pathway Tools and Resources at MaizeGDB

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Two maize metabolic network resources are available at MaizeGDB: CornCyc (http://corncyc.maizegdb.org), developed by the Plant Metabolic Network, and MaizeCyc (http://maizecyc.maizegdb.org), developed by Gramene. Both resources were created in collaboration with MaizeGDB, which performed literature-based manual curation. We compare strengths and weaknesses between the most recent versions of these resources and show how they can be complementary when interpreting transcriptomics data. Both resources are based on the B73 high-confidence gene models, and are well-integrated with the Pathway Tools platform that is equipped with powerful pathway visualization capabilities. A practical workflow is presented to demonstrate how maize researchers can upload gene expression data, paint metabolic pathways according to expression levels, and identify overexpressed pathways visually. Both resources are not only available online, but can also be downloaded for local use, along with Pathway Tools, which is freely available for the academic community. We also demonstrate a stand-alone computational tool in development: CycTools. CycTools can directly interact with Pathway Tools, and has enhanced search/import/export capabilities for annotations.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

P17

Genome-wide Appraisal of Loss-of–Function Variants in Zea mays.

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In the maize HapMap2 project (Chia et al. 2012) 55 million single nucleotide polymorphisms were identified in 103 lines representing domesticated Zea mays and its wild relatives. One of the striking findings of that study was that 7.5% of the 20,380 high-confidence genes exhibited in one or more lines SNPs for premature stop codon (nonsense) variation. As expected the frequency of nonsense mutations was greater in teosinte lines than in improved maize inbreds with a longer history of inbreeding. Among the lines sequenced in the HapMap2 project are the founders of the nested association mapping (NAM) population (McMullen et al. 2009). In the development and characterization of the NAM population it was noted that residual heterozygosity was higher in low recombination regions of the genome. The authors ascribed this phenomenon to the Hill-Robertson effect that predicts selection is less effective on alleles in repulsion in regions of limited recombination. This study follow up these two observations: 1) For the NAM founders and teosinte inbred lines the original HapMap2 sequence reads are used to call a broader range of loss of function (LoF) variation to include nonsense, start-loss, splice-site and small insertion/deletions that would result in frame-shift variation. Traditionally, LoF variants are regarded as rare and are of considerable scientific and clinical interest in the medical sciences. In the crop sciences, specific rare alleles have been reported that associate with grain size and yield. However, systematic analysis to understand the role LoF variants in agronomic traits in crop plants is lacking. 2) The distribution of LoF variation will be mapped across the genome and relationship between LoF variation and recombination examined.
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P18

Genomic features shaping the landscape of meiotic double strand break hotspots in maize

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Meiotic recombination is the most important source of genetic variations in higher eukaryotes. Recombination is initiated by formation of double-strand breaks (DSBs) in chromosomal DNA in early prophase of meiosis. The DSBs are subsequently repaired, resulting in crossovers (CO) and non-crossovers (NCO). In most eukaryotes, recombination events are not distributed evenly along chromosomes. Instead, regions with high recombination rates (recombination hotspots) are interspersed with regions of low recombination rates (coldspots). How specific chromosomal sites become recombination hotspots or coldspots is poorly understood. Here we show a genome-wide map of DSB hotspots that we generated in the maize B73 inbred line to elucidate factors determining the location of recombination events. We found that recombination in maize is initiated in all chromosome regions, including those known to be devoid of COs, such as centromeres and the pericentromeric regions. The vast majority of DSBs are formed in repetitive DNA, predominantly*Gypsy* retrotransposons. In contrast, only one-quarter of DSB hotspots are located in genic regions. Hotspots in repetitive and genic regions exhibit several distinct features, including the fact that only genic region hotspots contribute to the formation of COs. Our data also show that DSB hotspots in of maize exhibits characteristics not reported previously in mouse or yeast. Understanding recombination patterns will shed light on the mechanisms affecting dynamics of the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

P19

Genotyping by sequencing on a worldwide collection of maize inbreds, landraces and teosintes

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A large collection of maize inbreds, landraces and teosintes, approximately 45,000 samples in total, were sequenced using the genotyping by sequencing (GBS) protocol. We aim to discover and genotype 1.5 million SNP markers for all of the samples. In order to handle the large data set, our GBS bioinformatic pipeline was redesigned using a new strategy and the HDF5 format was applied. To improve the genotyping quality, especially the alignment which is the key for SNP calling, we developed genetic mapping approaches combined with machine learning algorithms to refine sequence alignment. From the pilot experiment evaluating the new strategy, we found: 1. Alignments are quite different between aligners; 2. Aligners differ in sensitivity and accuracy, but they are often complementary; 3. Each alignment should be viewed as a hypothesis; 4. Genetic mapping is needed to verify alignments. All together, this new strategy should significantly improve SNP discovery in complex genomes.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P20
Histone modification profiles are different between leaf and endosperm in maize

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As an important agricultural plant, maize has relatively bigger seeds compared to other cereal like wheat, rice and sorghum. Maize endosperm contains about 85% of starch, 10% protein and 5% lipid and has a highly coordinated developmental process at different levels of regulation. Previous genome-wide studies have illustrated the importance of DNA methylation and siRNA in controlling tissue-specific gene expression, but the understanding of regulation at the chromatin level is lacking. Here using next generation sequencing platform, we compared maize histone modification profiles between leaf and endosperm and found major differences that could account for differences in gene expression. H3K27ac is highly correlated with gene density in the leaf, but not in endosperm, indicating that it might have a significant role in gene activation during leaf development, when photosynthesis is pronounced. H3K4me2, H3K9me2, H3K27me2 and H3K9ac on the other hand are generally negatively correlated with gene density and there is only 10% overlap among their peaks. GO term genes are analyzed for enrichment in histone profiles between leaf and endosperm.

Funding acknowledgement: Selman Waksman Chair in Molecular Genetics

P21
Integrated network analysis and inference on maize endosperm development

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Revealing the regulatory relationship among genes and their products has been a long-standing challenge in molecular and computational biology. In order to better understand particular biological problems, it requires reverse engineering the complex regulations at DNA, RNA and protein levels, in dynamic time courses. Here we propose an integrated method for network analysis and inference, comprised of information theory and probability based algorithms. Various expression profiles of genes and/or gene products, which are measured under different circumstances including genomic variation, gene mutation, perturbation and environmental stimulation serve as input for our models. We illustrate this approach with applications on published data of maize endosperm development, i.e. high-throughput sequencing data of kernels at different days after pollination (DAP). First we create cross-referencing procedures for gene/protein expression profiles to exploit existing resources for gene annotation, transcription factor binding site, microRNA targets and well-studied metabolic pathways. Functional domains and motifs are also obtained by comparisons to protein families from the Pfam database using hidden Markov models (HMMs). The generated dynamic networks at different DAPs uncover interesting topological motifs in various hierarchical structures. We also develop a set of algorithms based on the basic Boolean dynamics, mutual information, and Bayesian probability, to infer potential regulatory relationships. For each predicted edge in the network graph, a confidence value is assigned based on the consensus of all incorporated models. Particularly, we calculate the conditional independence of gene/protein pairs, remove those irrational connections, and construct networks with weighted edges for confidence levels of prediction, based on information content or probabilities. The comparative network study on high-throughput imprintome data also shows distinctive patterns for paternally and maternally expressed genes.

P22

Large-scale proteomic and phospho-proteomic analyses of maize root tissues

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To contribute to a large-scale maize protein atlas we analyzed different root tissues of the inbred line B73. Primary roots (2-4 cm) were divided into four sections including (1) the meristematic zone (1,5-2 mm) including the root cap and (2) the elongation zone (2-4 mm). The elongation zone is delimited from the differentiation zone by emerging root hairs in differentiated epidermis cells. The differentiation zone was dissected into (3) cortical parenchyma including root hair bearing epidermis cells and (4) the stele. Multidimensional LC coupled with MS/MS identified almost 74.000 peptides across all 4 tissues representing more than 11.500 unmodified proteins. In addition, nearly 10.000 phosphopeptides, representing more than 2.900 phosphorylated proteins were identified. The analyses of these two proteomes in parallel allows for the identification of tissue-specific protein expression and phosphorylation changes in distinct tissues. Functional analyses using Mapman revealed that about 30% of the identified proteins and phosphorylated proteins were related to the categories protein metabolism and RNA. Motif analyses of phosphorylated proteins revealed the abundance of Ser (83%), Thr (16%), and Tyr (1%) phosphorylation. The identification of proteins and phosphorylations which are enriched in distinct root tissues helps to better understand maize root development and differentiation.

P23

Long non-coding RNAs responsive to nitrogen deficiency in maize leaves

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Long non-coding RNAs (lncRNAs) are adding a new layer of gene regulations as they are implicated in diverse biological processes responsive to bio- and abio-stresses. Nitrogen (N) is an essential and often limiting nutrient to plant growth. Previous studies have shown that the mRNA expression of numerous genes was regulated by nitrogen treatments, yet little is known about the non-coding elements controlling maize response to N. In this studying, we surveyed the lncRNAs, including nonpolyadenylated lncRNAs and cis-natural antisense transcripts, in B73 and Mo17 leaves at V5 stage under conditions of N-deficiency and N-sufficiency using deep sequencing and strand-specific libraries. Integrated with mRNA expression profiles and physiological evaluations, we discover that hundreds of lncRNA candidates and their adjacent annotated genes associated with N responses in maize. Furthermore, we attempt to reveal the function of these lncRNAs by constructing a co-expression network using the weighted correlation network analysis (WGCNA). Several lncRNAs were annotated as belonging to co-expressed genes enriched in various N-responsiveness related biological processes. We focused the genetic analyses on a subset of these lncRNAs that exhibit statistically significant expression difference between B73 and Mo17 after N deficiency. Our results illustrate the potential for regulatory roles of lncRNAs respond to N stress, which lay an important foundation for in-depth understanding the mechanisms involved in the plant responses to stress.

Funding acknowledgement: Natural Science Foundation of China (NSFC)

P24
Maize Pan-genome construction by short reads assembly

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In Zea mays it is estimated that only 50% of genomic content is held in common between lines due to tremendous haplotype diversity. A single reference assembly for maize cannot serve as a sufficient backbone to capture and describe this variation and is a limiting factor in understanding the genetic variation controlling traits. The maize pan-genome under development will be composed of the B73 reference assembly and novel sequences from other maize inbred lines. Our initial efforts are to identify novel haplotypes among a broad sampling of sequenced germplasms by conducting whole genome assembly of 30X Illumina reads. At this depth, high repeat content in maize will challenge existing methods for de novo assembly. Simulations using the ALLPATHS-LG recipe to assemble 30X reads sampled from the B73 reference resulted in ~82% coverage of annotated genes. We are using sequence data for the inbred B97 to further prototype pipeline construction. For the test line B97, we have ~36X paired end reads (180bp inserted size), 5X 2kb and 5kb mate pair reads. Because different assembly algorithms have different advantages, we have adopted a meta-assembly strategy. Primary assemblies by ALLPATHS-LG and SOAPdenovo were subjected to contigs merging and re-scaffolding by SSPACE. Using this strategy, ~30% of the genome could be assembled with ~72% of genic space covered in scaffolds. After aligning to the B73 reference, ~19.78 Mb of novel sequences were identified. We also detected 3,484,475 SNPs and 2,410,907 indels. Of these SNPs, a total of 2,292, 960 sites were shared in maize HapMap2, and the identical genotype rate was 95.92%. We are also using a genetic mapping method to anchor novel sequences relative to the B73 physical map. The accuracy rate of this genetic mapping method was tested by anchoring 1,000 B73 genes, which showed to be 77.6%. This work was funded by NSF awards #1238014 and #1127112.

Funding acknowledgement: National Science Foundation (NSF)

P25
PIGD: A database for intronless genes in the Poaceae

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Intronless genes, as a prokaryotic feature, are widespread and unequally distributed in the eukaryotic tree and represent an important resource for the study of the evolution of gene architecture. In this study, we present Poaceae Intronless Genes Database (PIGD), a user-friendly web interface to explore the information of intronless genes from different plants. Five Poaceae organisms, *Sorghum bicolor*, *Zea mays*, *Setaria italica*, *Panicum virgatum* and *Brachypodium distachyon* were included in the current release of PIGD. Gene annotations and sequence data were collected and integrated from different databases. The primary focus of this study was to provide gene descriptions and gene product records. In addition, functional annotations, subcellular localization prediction and taxonomic distribution were reported. PIGD allows users to readily browse, search and download data. BLAST and comparative analyses are also provided through this online database, which is available at http://pigd.ahau.edu.cn/.

Funding acknowledgement: National Science Foundation (NSF)

P26

RNA sequencing reveals the complex regulatory network in the maize kernel

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RNA sequencing can simultaneously identify exonic polymorphisms and quantitate gene expression. Here we report RNA sequencing of developing maize kernels from 368 inbred lines producing 25.8 billion reads and 3.6 million single nucleotide polymorphisms. Both MaizeSNP50 BeadChip and the Sequenom MassArray iPLEX confirm a subset of high-quality SNPs. In these SNPs, we have mapped 931,484 to the gene regions with a mean density of 40.3 SNPs per gene. The genome-wide association study identifies 16,408 expression quantitative trait loci. A two-step approach defines 95.1% of the eQTLs to a 10 kb region, and 67.7% of them include a single gene. The establishment of relationships between eQTLs and their targets reveals a large-scale gene regulatory network, which include the regulation of 31 zein and 16 key kernel genes. These results will contribute to our understanding of the kernel development and to the improvement of maize yield and nutritional quality.

P27

Systems biology approaches for integrating datasets identifying genes related to iron nutritional quality in maize

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Previously described linkage studies identified more than 10 QTL controlling iron nutritional quality (e.g. bioavailability and density) in the IBM RIL population. However, as is typical of linkage studies, these 10 QTL contain over 6,400 genes making the identification of causal variants difficult. To complement these QTL, non-targeted metabolite profiling by mass-spectrometry was used to characterize the grain metabolome in the Goodman Diversity Panel. Modules of co-varying metabolites were used as features for genome-wide association mapping. Combining metabolite module-genome associations with previously discovered iron bioavailability QTL, however, is problematic. Integration is complicated by the fact that the metabolite features are largely anonymous and significant SNPs produce thousands of candidate genes. Furthermore, few metabolite module associations fell strictly within the 10 iron QTL. Here we show that global co-expression networks provide a basis for integrating these data to identify high-confidence loci controlling iron bioavailability; guilt by association relationships can be leveraged to better resolve candidate gene lists produced both by linkage mapping and metabolomic approaches.

Gene co-expression networks describing both developmental B73 tissues and diverse Zea genotypes were constructed. Using the coexpression networks as a statistical framework, we integrated all three datasets and identified densely co-expressed subnetworks of genes within QTL regions that were also linked to metabolite features. Integration by transcriptional co-regulation provides an intuitive way to validate candidate genes produced by linkage and metabolite profiling but also provides a basis for statistical inference on the likelihood of co-occurrence of genes linked to a biological process. Subnetworks discovered by this process were subjected to bootstrapping analysis, demonstrating that it is unlikely that such coherent subnetworks would be discovered by chance. Using this integrated approach, we were able to reduce our candidate list by two orders of magnitude leaving us with a tractable number of genes for further interpretation.

Funding acknowledgement: National Science Foundation (NSF), University of Minnesota Interdisciplinary Informatics Initiative

P28

The *iPlant Collaborative*: A Unified Cyberinfrastructure for Plant Science

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The iPlant Collaborative (NSF #DBI-1265383) (www.iplantcollaborative.org) provides a community platform servicing diverse domains of plant research. From data hosting and storage to analysis and discovery, this NSF funded resource is openly available to investigators navigating the challenges and pursuing the promises of data-driven biology.

Research in biology increasingly depends on data-intensive methods and complex computational analyses that span a range of investigational domains - from genomics and transcriptomics, to phenotyping and ecology. iPlant cyberinfrastructure (CI) services a broad range of biological research questions by providing a unified platform for the storage, sharing, and analyses of large datasets – from genomes to image data, and beyond. Additionally, the variety of iPlant tools and access points cater to every level of user, from bench-biologists to bioinformaticians; removing many of the barriers researchers face in managing and sharing large data sets and conducting sophisticated computational analyses.

iPlant CI is made of several interconnected platforms including: **Discovery Environment** iPlant's web-based platform for managing data, and running analyses is a scalable and extensible platform featuring hundreds of commonly used bioinformatics tools accessible through a simple sleek interface; **Atmosphere** iPlant cloud services for harnessing on-demand computational power and 1-click access to a custom computing environment; **Data Store** iPlant's data storage system furnishes great flexibility and control over data; fast uploads/downloads, and terabytes of storage; **Foundation API** underlies much of iPlant CI, and can be accessed directly by developers.

Funding acknowledgement: National Science Foundation (NSF)

P29

The N-terminus of the ROUGH ENDOSPERM3 splicing factor is necessary for splicing U12-type introns

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The splicing of introns from pre-mRNA transcripts is catalyzed by two different spliceosomes in metazoans and higher plants. The major spliceosome removes U2-type introns, which are the most common type of intron. The minor spliceosome removes the rarer U12-type introns. The U2AF^{35}-related protein is a core splicing factor that is necessary for the removal of both types of introns in human cells, but the splicing factor plays different roles in each spliceosome. The domains of the U2AF^{35}-related protein that are necessary for the function of each spliceosome remain unknown. Here we show that an insertion/deletion mutation in the N-terminus of the ROUGH ENDOSPERM3 splicing factor, a maize U2AF^{35}-related protein ortholog, selectively impairs the splicing of U12-type introns in the maize *rough endosperm3* (*rgh3*) mutant. RNA-seq analysis of *rgh3* and wild-type seedlings was used to identify splicing defects in the mutant. No U2-type introns showed differential splicing in this analysis. However, 43% of the readily identifiable U12-type introns exhibit splicing defects in *rgh3* mutants . The *rgh3* splicing defects include intron retention and cryptic splice site activation, and preferentially affect genes related to cell cycle and cytoskeletal organization. During seed development, *rgh3* endosperm cells show cell type differentiation defects and retain the ability to proliferate in tissue culture until late in seed development, as shown in a previous study. The *rgh3* mutant causes an insertion-deletion polymorphism that alters the N-terminal acidic domain of the protein indicating that this domain is necessary for normal U12 splicing.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), University of Florida Genetics Institute, University of Florida Plant Molecular and Cellular Biology Program

P30

The structure and evolution of *mTERF* gene families

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Mitochondrial transcription termination factor (mTERF) genes comprise a large family with important roles in regulating organelle gene expression in plants. The evolution and biochemical functions of the plant *mTERF* genes remain unclear. Here we analyzed the *mTERF* gene families contained within the whole genomes of algae, mosses, ferns, gymnosperm and flowering plants. The results revealed that there are much more *mTERF* genes in higher plants than in lower plants. Phylogenetic analysis indicated that a mitochondria-targeting higher plant-specific clade (M-class) accounts for the extension of *mTERF* gene family in higher plants to a substantial extent and the appearance of M-class*mTERF* genes is closely associated with diversity of mitochondrial genomes. As a case in maize genome, 31 potential *mTERF* genes were identified based on comprehensive database search, and most of them were targeted to mitochondria or chloroplast. *mTERF* genes were divided into nine main groups by phylogenetic analysis in maize and groups VIII and IX represented the mitochondrial and species-specific clade belonging to M-class. The tandem and segmental duplication both contributed to the expansion of *mTERF* gene family in maize genome. Comprehensive expression analysis of these genes using microarrays data and RNA sequencing data, revealed that these genes exhibit a variety of expression patterns in differentially developed tissues. Environmental stimulus experiments revealed differential up-regulation or down-regulation expression of maize *mTERF* genes in seedlings exposed to light/dark, salts and plant hormones, respectively. These results will be useful for elucidating the roles of *mTERF*s in the growth, development and stress response in higher plants.

Funding acknowledgement: National Natural Science Foundation of China,National High Technology Research and Development Program of China (863 Program),National Basic Research Program of China (973 Program)

P31

The UniformMu transposon resource for functional genomics

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The UniformMu reverse genetics resource continues to grow with addition of new maize transposon insertion mutants. The UniformMu population utilizes the Robertson's Mutator transposon for systematic, insertional mutagenesis of the maize genome thus enabling analysis of genotype-phenotype relationships in a uniform inbred genetic background. The locations of germinal transposon insertions in thousands of maize lines are precisely mapped in the maize genome using the high-throughput Mu-seq sequencing method. Mutants can be searched on-line by sequence through the "Popcorn" portal at MaizeGDB.org, and seed stocks are available free of charge through the Maize Genetics Cooperation Center. Currently, the resource contains over 52,000 germinal transposon insertions mapped in 8,832, F3 seed stocks including insertions in at least 15,400 genes. For over half of these genes (8,500), two or more independent insertion alleles aiding confirmation of genotype-phenotype relationships. In the course of creating the UniformMu resource, we have developed the Mu-seq method that enables efficient high-throughput sequencing, identification, and mapping of transposon insertions in large numbers of maize plants. The location of each insertion is precisely defined. Thus, information about the context of each insertion is available to aid users in selection of mutants that have high probability of disrupting gene function.

Funding acknowledgement: National Science Foundation (NSF)

P32

Transcriptional profiling of lluteño maize under salt stress and excess of boron, a northern Chile maize highly tolerant to the abiotic stress

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Abiotic stresses have become an integral part of crop production because they severely compromise plant growth and development. Salt and boron abundance in soil and irrigation water are important determinants of agricultural productivity. Salinity reduces the ability of plants to take up water resulting in reduced growth rates along with metabolic changes. Excess of boron reduces growth in shoots and roots, alters metabolism, lowers leaf chlorophyll content, and reduces photosynthetic rates among other detrimental conditions.
The Lluta valley (Arica, Chile) presents soil and irrigation water with high salt and boron concentrations and other elements, around four to six times greater than soils used for commercial production respectively. Lluteño maize (Z. mays cv. amylacea), is a variety of sweet corn grown in the Lluta valley, is selected using traditional breeding by local farmers to tolerate high salinity and excess of boron. Due to the salinity and boron tolerant characteristics, lluteño maize has become a good system to explore the maize biodiversity as a potential source of target genes for future crop breeding programs.

Our objective was to identify, compare and characterize genes that respond to stress induced by high NaCl and boron concentration in lluteño maize, throught a global gene expression profile.
Maize seedlings were grown in a hydroponic culture for 7 days, and then were stressed by NaCl (150 mM) and boron (30 ppm). Leaves and roots samples were collected at 3 and 96 hrs after stress for gene expression analysis.

A total of 1632 and 161 genes responded to NaCl and boron respectevely, which were involved in various metabolic and signal transduction networks. Responsive genes with differential expression under stress compared to control were validated by qRT-PCR. Currently, we are performed heterologous overexpression of some promising candidate genes in Arabidopsis. Our results could facilitate breeding of salt and boron tolerant maize varieties.

Funding acknowledgement: Fondecyt 11100492, FIC-Regional BIP-30110588-0, Convenio de Desempeño MECESUP-2.

P33

When Alternative Splicing Meets Whole Genome Duplication and Gene Body Methylation

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Alternative splicing (AS) is a mechanism to regulate gene expression and increase protein diversity. Whole genome duplication followed by functional divergence of duplicated genes has made major contributions to protein and species diversity within plant lineages. After genome duplication, paralogous genes may acquire novel functions (neofunctionalization) or partition existing functions between duplicated genes (subfunctionalization). However, it is unknown whether these processes acting at the mRNA splicing levels. Maize and sorghum are ideal species to test these hypotheses due to extensive genomic resources in both species and because the maize lineage experienced a whole genome duplication event 5-12 million years ago, subsequent to divergence with sorghum. Our research suggest that the degree of AS differs substantially between maize and sorghum following duplication, frequent changes in AS potential between two maize paralogs relative to sorghum have occurred. Based upon current results neo-functionalization seems to be the dominant fate for the mRNA splicing rather than subfunctionalization after whole genome duplication.

In additional to conventional role of DNA methylation in silencing gene expression, methylation sometimes also exists within genes that are expressed, a phenomenon called gene body methylation. Gene body methylation has been observed in many eukaryotic genomes but its biological function remains unclear. Recent studies in invertebrates and mammals support the idea that gene body methylation is correlated with AS. We are examining this hypothesis in maize and hope to determine which type of gene body methylation (CG, CHG or CHH) is correlated with AS. To this point, we have found that AS is associated with CG and CHG gene body methylation, whereas CHH methylation is not. We are currently processing additional RNA-Seq data and finalizing our bioinformatics results.

P34

A CRISPR/Cas-based Toolkit for Maize Multiplex Genome Editing, Multigene Interference, and Multigene Activation

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We developed a CRISPR/Cas-based binary vector set for maize multiplex genome editing, multigene interference, and multigene activation. Each vector carries maize codon–optimized Cas9 or one of its derivatives including Cas9D01A, dCas9-VP64 and dCas9-KRAB, driven by maize ubiquitin 1 promoter. Each vector also contains a guide RNA expression cassette, in which the guide RNA is driven by rice U3 gene promoter. We set up methods to assemble two or more gRNAs in only one cloning step and with high efficiency. With the vector set and the methods, users could generate rapidly a CRISPR/Cas binary vector carrying Cas9 and one to multiple gRNAs targeting one to multiple genes. We validated the set using maize protoplasts, exhibiting high efficiency. We demonstrated that the rice U3, wheat U3 and Arabidopsis U6-26 gene promoters could drive the expression of guide RNA in maize efficiently. Comparison of mutation efficiencies in maize protoplasts determined that the maize codon–optimized Cas9 performed much better than its human codon–optimized counterpart did. The set that facilitates transient or stable expression of CRISPR/Cas or a derivative system in maize could provide a platform for maize functional genomics and biotechnological applications.

Funding acknowledgement: the National Basic Research Program of China (2012CB114200), the National Science Foundation of China (31070329), and the National Transgenic Research Project (2011ZX08009).

P35

A semi-in vitro system for studying genetic compatibility

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Several genetic systems control cross compatibility in maize. The Ga1 locus is the most widely studied and is responsible for exclusion of dent corn pollen from popcorn varieties. Other loci including Tcb1 and Ga2 have been identified in teosinte. It may be possible to use these systems to preserve grain purity in different market classes of corn. In order to better understand the biochemical function of cross compatibility in maize, we developed a semi-in vitro system. In this system, the kinetics of pollen germination are monitored spectrophotometrically in 96-well plates. Silk extracts from different sources can be added to the pollen germination reactions to observe the effect on pollen germination kinetics. We have reproduced the ga1 pollen - ga1 silk compatible reaction as well as the ga1 pollen - Ga1-S incompatible reaction in this way. This system will allow biochemical characterization of the pollen compatibility reaction and complement efforts to identify genes involved in cross compatibility.

Funding acknowledgement: United States Department of Agriculture (USDA)

P36

Alternative splicing events in two maize lines under herbicide stress conditions

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Plants, as sessile organisms, must adapt their growth and metabolic style to a changing environment. Splicing is one of the mechanisms which play an important role in plant adaptation and is an additional element of fitness benefit adjusted to the limited capacity of genome size.
Studies of splicing and its role in diverse aspect of cell biology, pathology and stress response, has remained undescribed for many plant species, including maize. Through the mechanism of alternative splicing, exons from primary transcripts (pre-mRNA) with multiple introns may undergo ligation in many different ways generating multiple proteins from single gene. This process can affect mRNA stability and translation efficiency as well as activity, cellular localization, regulation and stability of coding protein.
For better characterization of alternative splicing role in plant herbicide stress response, we sequenced transcriptomes of two maize breed lines – sensitive and tolerant to herbicide RoundUp. We used Illumina next-generation sequencer Genome Analyzer IIx and we conducted pair-end sequencing. As a result we obtained 35 to 76 mln 50nt reads per sample.

Using bioinformatics tools such as BowTie, TopHat, Cufflinks, Cuffdiff and CummRbund we managed to identify between sensitive and tolerant maize line. We also managed to identify different types of splicing events with java script.

Funding acknowledgement: Ministry of Science and Higher Education (Republic of Poland), National Science Centre (Poland)

P37

An integrative omics strategy for deciphering the molecular regulation network in maize roots under chromium exposure

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Soil contamination by chromium (Cr) has become an increasing problem as a result of extensive industrial activities around the world. Excessive chromium, especially hexavalent Cr, negatively affects the growth and productivity of plants. Although it is proposed that plant could adjust metabolisms to cope with Cr stress by reprogramming gene expression, direct evidence at molecular level is lacking. In order to better understand the mechanisms underlying the plant response to Cr exposure, in the present study, the time-course changes in the protein expression, metabolites and ionome induced by Cr exposure were analyzed in maize roots. We use a combination of two-dimensional gel electrophoresis and ITRAQ quantitative proteomics technology to monitor the dynamic change in maize proteome, and a batch of differentially expressed proteins were identified. Functional classification of these proteins identified a set of biological processes that were affected under Cr stress, including ROS detoxifying, hormone signal transduction, protein synthesis, folding and degradation, RNA processing, vesicle transport, transmembrane transport, energy metabolism, chromatin mediated epigenetic regulation, and cell division. The early signal transduction pathway was also investigated using quantitative phosphoproteomics method, and hundreds of phosphorylation sites regulated by Cr stress were mapped. We further discussed the relation between the alteration in proteins, metabolites and ions. Base on our results, a model on the molecular regulation network in maize roots under chromium exposure was proposed. Our finding provides interesting candidate genes for further research with an aim to manipulate the Cr tolerance in maize using genetic engineering.

Funding acknowledgement: the National High Technology Research and Development Program of China, the National Training Programs of Innovation for Undergraduates

P38

Analysis of Stalk Fiber Quality of Maize

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Maize is the most important cereal and forage crop worldwide. Maize stalk digestibility and lodging resistance were well known as the major factors affecting yield and forage quality in maize. While cellulose (CELLU), hemicellulose (HCELLU) and acid detergent lignin (ADL) as the major components of maize stalk significantly correlate with lodging and digestibility. So the objective of this study was to explore the genetic basis of these traits. Materials employed 200 lines of recombinant inbred lines (RIL) population derived from B73×By804. Near-infrared reflectance spectrometry was used to analyse phenotypic traits in diffident environments. Five additive-effect QTLs were detected for ADL, located on chromosome 8 and 10, which accounted for <10% of phenotypic variation. Ten additive-effect QTLs were detected for CELLU, located on chromosome1,6,7,9,10. QTLs on chromosome 6,7,10 Shared common flanking markers or had overlapping confidence intervals in different environments. Among all these QTLs, the QTL on chromosome 7 (Phi091-atf2) had the largest percentage (12.44%) of contribution to phenotypic variation. Seven additive-effect QTLs were detected for HCELLU, located on chromosome 5,6,7,9,10, the QTL on chromosome 5(umc2115- umc2036) having the largest effect could explain 11.39% of the phenotypic variation. And on chromosome 1 and 4, one pair of epistatic QTL was identified, which the percentage of contribution to the phenotypic variation was 11.26%. For stalk fiber quality traits, CELLU and HCELLU shared common flanking markers on chromosome 7(left flanking marker atf2) and 9(left flanking markers phi032 and umc1258). CELLU, HCELLU and ADL shared common flanking markers on chromosome 10(left flanking marker umc1506). These results indicated that a lack of major QTL for stalk fiber quality traits and these traits may be controlled by multiple genes with small effects; The corresponding results obtained from correlation analysis and QTL mapping suggested the presence of pleitropism or linkage between genes.

Funding acknowledgement: National Natural Science Foundation of China (NSFC）

P39

Auxin effects on maize seed transcriptome during the early stages of endosperm development

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In angiosperms, the endosperm is the primary nourishing tissue that provides nutrients for seed germination and in monocots it represents the main constituent of the seed. Cereal seed, and in particular maize seed, could be used as a model for the study of the endosperm tissue. To discover the genes involved in early seed development we performed a time course experiment of the transcriptome of the maize endosperm at 8, 12 and 16 DAP. Endosperm transcript profiling was extended to a mutant impaired in auxin biosynthesis, *defective endosperm 18* (*de18*), to study the hormone-dependent transcriptional network and in particular the auxin effect on transcriptome. The number of transcripts aligned to the reference genome progressively decreased from 8 to 16 DAP and the majority of them were up-regulated in the earliest stage. This evidences a more dynamic transcriptional regulation at 8 DAP, as expected for endosperm that is a tissue that undergoes cell death at maturation. Gene Ontology (GO) analysis of annotated genes showed that these genes were enriched in biological process terms such as cellular and metabolic processes, regulation of biological process, localization and response to stimulus. As regards the molecular function category, the most representative belong to the binding and catalytic activity terms. The main GO term found up regulated during development was the nutrient reservoir activity that is in agreement with the storage accumulation function of endosperm. The regulation of RNA metabolic process, transcription and signaling were the main GO terms down regulated together with the RNA transport, mRNA surveillance and biosynthesis of secondary metabolites pathways. DEGs belonging to hormones signaling pathways, cell cycle and epigenetic effectors were evidenced and validated by real time PCR and *in situ* hybridization. A morphological description of the mutant and wild type together with the *in situ* hybridization of the most interesting genes will allow localizing genes related to early endosperm development.

Funding acknowledgement: FIRB RBFR08UG7J, Ministry for Education and University (MIUR), Italy

P40

BAC/BIBAC resources and services for functional and comparative genomics studies of maize

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BAC (bacterial artificial chromosome) and BIBAC (binary BAC) resources are important tools for positional cloning, whole or targeted genome sequencing, comparative mapping and large DNA fragment transformation. To facilitate functional and comparative genomics studies in maize and make the best use of the maize reference sequence, we constructed BAC libraries for the maize inbred lines Zheng58, Chang7-2 and Mo17, and the maize wild relatives *Zea mays* ssp. *Parviglumis* and *Tripsacum dactyloides*, and constructed BIBAC libraries for the maize inbred line B73 and sorghum land race Nengsi-1. The seven ZMAP (Zea Map Alignment Project) BAC/BIBAC libraries all have a high quality. The BAC/BIBAC vectors used facilitate transfer of large intact DNA inserts from BAC clones to the BIBAC vector and functional complementation of large DNA fragments. All these resources are available to the public through our website (http://GResource.hzau.edu.cn). Besides, we also provide services for custom BAC library construction, PCR screening of BAC/BIBAC libraries, BAC sequencing, physical mapping and BAC end sequencing.

Funding acknowledgement: National Basic Research Program of China (the “973” project, 2009CB118404)

P41

Bulked segregant analysis (BSA) to map 120 *rough endosperm* (*rgh*) seed mutants from the UniformMu transposon-tagging population

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Conventional genetic analysis of seed lethal, *defective kernel* mutations is hampered by the similarities in mature kernel mutant phenotypes and the requirement to maintain the mutations as segregating heterozygous stocks. Genetic map positions for seed mutant isolates enable targeted genetic complementation tests. We mapped 120 *rough endosperm* (*rgh*) seed mutants from the UniformMu transposon tagging population. The *rgh* mutants develop a rough, etched, or pitted seed surface at maturity, while UniformMu harbors Mutator transposons in a W22 inbred genetic background. Over 140 *rgh* mutant isolates were crossed to B73 and Mo17 inbreds to generate F_2 mapping populations. Greater than 220 Bulked Segregant Analyses (BSA) of 60 *rgh* mutant seed pools were genotyped using 143 to 144 distributed SNP markers. The BSA identified map locations for 120 isolates. Of these, 26 mutants were mapped with both a B73 and Mo17 mapping population, while 94 were mapped with a single BSA pool. Map positions were confirmed using individual recombinants for 22 mutant isolates. Based on these results, we estimate that the BSA map positions are accurate 90% of the time and that the 120 mutants represent at least 60 genetic loci. These results will allow more rapid assessment of transposon insertions for co-segregation with *rgh* mutant phenotypes.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), HHMI

P42

CACTA-like transposable element in *ZmCCT* attenuated photoperiod sensitivity and accelerated the postdomestication spread of maize

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The postdomestication adaptation of maize to longer days required reduced photoperiod sensitivity to optimize flowering time. We performed a genome-wide association study and confirmed that *ZmCCT*, encoding a CCT domain-containing protein, is associated with the photoperiod response. In early-flowering maize we detected a CACTA-like transposable element (TE) within the *ZmCCT* promoter that dramatically reduced flowering time. TE insertion likely occurred after domestication and was selected as maize adapted to temperate zones. This process resulted in a strong selective sweep within the TE-related block of linkage disequilibrium. Functional validations indicated that the TE represses *ZmCCT* expression to reduce photoperiod sensitivity, thus accelerating maize spread to long-day environments.

Funding acknowledgement: 2009CB118402 and 2011CB111509 from the National Basic Research '973' Program of China, 2012AA10A306&2012AA10A307 and 2012AA101104 from the National High-Tech and Development Program of China

P43

Characterization and cloning of a *slcd* mutant simultaneously affecting seed and leaf color

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Mutant, an important material for both classical genetics and functional genomics research, plays a significant role in the functional annotation of genes and the interpretation of biochemical and metabolic pathways in plants. In this study, a seed color mutant of maize, *slcd* (seed and leaf color defective), was identified naturally in a line in Zong3 × Yu87-1 recombinant inbred line population. The mutation is characterized by the light yellow grain and the albino plant, which results to the death of the whole plant. Genetic analysis of the mutant showed that the two types of phenotype, normal and mutation, in the segregating populations derived from the heterozygotes, fit the ratio of 3∶1. This result indicates that *slcd* mutant is controlled by recessive alleles. Using positional cloning, we isolated the gene responsible for the *slcd* mutant. A 7608-bp insertion of a gypsy-like LTR retrotransposon was detected in intron 8 of *slcd*, which results in the loss of function of this gene by producing alternative transcript splices, and subsequently leads to the *slcd*phenotype. Further experiments will be performed for functional validation of *slcd*.

Funding acknowledgement: National Natural Science Foundation of China (NSFC)

P44

Characterization of Maize Genes involved in ABA Signal Transduction

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Abscisic acid (ABA) contributes to both plant development and plant response to the environment. Its functionality includes regulating the onset and maintenance of seed dormancy, and signaling response to changes in plant water status. Its central role in plant water relations makes ABA perception and signal transduction ideal targets for altering plant responses to changes in water status. The recent discovery of the ABA receptor and recapitulation of an ABA signal transduction cascade in Arabidopsis makes it possible to contemplate strategies to leverage ABA signaling for the purpose of crop improvement. One approach is wholesale or targeted over expression of one or more members of the ABA signaling cascade. A risk is that direct intervention in the signaling cascade will compromise crop performance. Another approach is to develop modified ABA receptors that respond to ligands other than ABA. This affords the opportunity manipulate specific ABA signaling events, by adding a signaling switch without disrupting the natural ABA signal transduction cascade. A thorough understanding of key signal transduction components is necessary to begin this work. We defined and have begun characterizing the maize ABA receptor and protein phosphatase 2C (PP2C) gene families. Data to date indicate there are 12 functional ABA receptors and 12 PP2C candidates. Overlapping expression profiles among gene family members indicate the presence of functional redundancy that has been reported in other systems. The objective of this work is to show that ABA signal transduction can be manipulated to improve crop performance.

P45

Characterization of population structure of a set of sweet corn inbreds by developing SNP markers based on SLAF-seq

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We have introduced and developed more than 400 sweet corn inbred lines in twenty years. A diversity set including fifty elite lines was screen out from these lines based on the pedigree and Agricultural performance. In order to elucidate the population structure and haplotype of the set of inbreds, specific length amplified fragment sequencing (SLAF-seq), an efficient method of large-scale genotyping, was employed in this study to obtain sufficient SNP markers for haplotyping. In total, 175,049 SLAFs were acquired, with average depth 3.63. These SLAFs, 56.29% of which is polymorphic, is well-distributed on chromosome. Totally 569,054 SNP markers were discovered based on those polymorphic SLAFs. More than 900 haplotype blocks (block size >1kb) were estimated in Haploview. 84% of blocks are in the range of 1~30kb. Subsequently, according to distance-based clustering and model-based estimation, above 50 accessions were divided into two subgroups in correspondence with tropical and temperate ecotypes. Twenty lines were clustered into the first subgroup, including lines which come primarily from tropical or subtropical area, e.g., Taiwan, Thailand. Thirteen lines were clustered into the second subgroup, including lines which come primarily from temperate area, e.g., Chinese Mainland, America and Japanese. Others consist of some mixed lines. The results may provide clues for elucidating actual genetic constitutions of germplasm and selecting parents in sweet corn breeding.

Funding acknowledgement: Presidential Foundation of the Guangdong Academy of Agricultural Sciences (No. 201010), Natural Sciences Foundation of Guangdong Province (No. 8251064001000002), Guangzhou Science and Technology Program (No. 2012Y2-00007).

P46

Cloning and characterization of the male sterile 1 (ms1) gene in maize

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The application of the male sterile line is an effective approach to produce corn hybrids. It not only saves the labor of artificial emasculation and decreases the cost of seed production, but, most importantly, improves the quality of hybrid seed. In this study, ms1 was backcrossed with Z58 to generation a BC2F1 mapping population. Using the genome-wide IDP markers developed based on the sequence differences between B73 and Mo17, we have mapped ms1 to a small region on chromosome 6. The ease to execute map-cloning in maize has greatly benefited from the completion of maize genome. In addition the next-generation sequencing has been proved to a efficient technology to accelerate gene discovery. By deep-sequencing a pool of ms1 mutant and compared to its sibling control, we expect to unravel the casual gene underlying the ms1 mutant. Cloning and characterization of more male sterile mutants will be valuable for both basic biology and plant breeding

P47

Combined small RNA and degradome sequencing reveals microRNA regulation during immature maize embryo dedifferentiation

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Genetic transformation of maize is highly dependent on the development of embryonic calli from the dedifferentiated immature embryo. To better understand the regulatory mechanism of immature embryo dedifferentiation, we generated four small RNA and degradome libraries from samples representing the major stages of dedifferentiation. More than 186 million raw reads of small RNA and degradome sequence data were generated. We detected 27 (microRNA) miRNA families, including 100 known miRNAs as well as seven novel miRNAs and 13 new members of conserved miRNA families. In total, we identified 64, 85 and 77 differentially expressed miRNAs (DEMs) in the stage Ⅰ, Ⅱ, Ⅲ samples, respectively, compared to the control. However, only approximate 10% of the DEMs was regulated by more than five-fold during dedifferentiation. A total of 100 genes were identified as the targets of 18 DEM families. This group of targets was enriched in members of four significant pathways including plant hormone signal transduction, antigen processing and presentation, ECM-receptor interaction, and alpha-linolenic acid metabolism. The hormone signal transduction pathway appeared to be particularly significant, involving 21 of the targets. We further showed that inducing the auxin pathway and suppressing the gibberellin pathway were both necessary for dedifferentiation. Moreover, 12 of the DEM targets were located in the regions of quantitative trait loci controlling dedifferentiation, as identified in our previous study, and ten of them were among the targets of the most significant DEMs. Our results provide important information regarding the regulatory networks that control immature embryo dedifferentiation in maize.

Key Words: dedifferentiation, degradome sequencing, immature embryo, maize, miRNA regulation, small RNA sequencing, target.

Funding acknowledgement: National Science Foundation (NSF)

P48

Construction and Characterization of a Bacterial Artificial Chromosome Library of Maize Inbred Line Qi319

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Zea mays L. is the crop that has had the highest area and yield in China since 2012. The incidence and prevalence of corn diseases affecting maize yield, especially southern corn rust and rough dwarf disease which broadly distributed southeast of China and Yellow-Huai River maize zone, cause great losses. The bacterial artificial chromosome (BAC) cloning system is an invaluable tool in the cloning of disease resistance genes and in conducting structural and functional analysis.
A BAC library for Qi319, the key source for disease-resistant maize breeding in China, was constructed in this study. Based on optimisation conditions of high-molecular weight DNA extraction and partial digestion of DNA, the digested fragments were ligated into CopyControl pCC1. The library contains 270,720 clones with an average insert size of 90 kb. Based on a haploid genome size of 2300 Mb, the coverage of the library is about 10.43 genome equivalents, providing 99.99% possibility to isolate any maize gene or sequence in the library. We obtained 8, 14 and 15, 11 positive clones by PCR screening using primer pairs linked to the resistance genes of maize southern rust and rough dwarf, respectively.
We constructed the BAC library of maize inbred line Qi319 and tested its quality. The results indicate the library can satisfy the requirements to recover specific sequence. In the future, the BAC library will serve as both a giant gene resource and an invaluable tool for map-based gene isolation, physical mapping and comparative genome analysis.

Funding acknowledgement: Shandong Provincial Natural Science Foundation, China

P49

Coordination between methionine storage and cysteine and methionine biosynthesis in maize

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In plants, 5'-adenylylsulfate (APS) reductase and serine acetyltransferase are hypothesized to be key control points in sulfur (S) assimilation leading to the synthesis of the S amino acids Cys and Met. Maize expresses a variety of different seed storage proteins and preliminary research suggested that the expression of the zeins with high Met content might be significantly impacted by the rate of Cys and Met synthesis. To explore this hypothesis three different genetic engineering approaches were used to increase Cys and Met synthesis. Deregulation of S assimilation was attempted by heterologous expression of three different S reduction or assimilation enzymes under control of the RbcS promoter including: Pseudomonas aeruginosa 5'-adenylylsulfate reductase (PaAPR); Escherichia coli 3'-phospho-5-'-adenylylsulfate reductase (EcPAPR); or Arabidoposis serine acetyltransferase (AtSAT1). The transgenic lines were created in the maize BA hybrid, and were backcrossed to B73 for two generations (T2). All three transgenic approaches significantly increase S assimilation as evidenced by the accumulation of glutathione, a major end-product of the S-assimilation pathway. In addition, expression of the heterologous enzymes resulted in accumulation of the high Met 10 kDa zein, raising total Met up to 6 fold. The effect was evident in a variety of maize genetic backgrounds including BSSS53, A654, and Mo17. The highest increase in kernel Met was observed in the AtSAT1 and EcPAPR lines. Although, PaAPR lines also showed the increase, these lines were characterized by negative vegetative growth impact. Our results confirm that the control of S assimilation profoundly influences the accumulation of high Met-content zeins. Further work will explore the physiological and genetic interactions between nutrient assimilation and control of seed storage protein expression in maize.

Funding acknowledgement: Selman A. Waksman Chair in Molecular Genetics, New Jersey Agricultural Experiment Station, National Science Foundation, China Scholarship Counsel (CSC)

P50

Cytological Study and Fine Mapping of the Male Sterile Gene ms6044

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Maize male sterile mutant ms6044 was conditioned by a single recessive gene. Cytological studies showed that the male sterile spikes were entirely abortive in the very early developmental stage and there was no pollen grain in the mature anthers. Further observation indicated that the development of sterile anthers became abnormal at 2mm and ceased to elongate at 3.2 mm in length, which was about 60% of the full length of the normal anther. In addition, meiosis did not occur in the sterile anther, while other structures of anther and the female counterpart were normal. To fine map the *ms6044* gene, BC1F1 and F2 mapping population were constructed from crosses of ms6044 mutant inbred line and Zhen58 and B73 fertile lines. A total of 7000 individuals were screened and the *ms6044* was mapped into an interval of 140k flanked by the markers k268 and k119 on chromosome 1. Three candidate genes seemed interesting in this region. However, ms6044 mutant and the wild type had identical ORFs for two of the three candidate genes. Comparing with RT-PCR result and the Sequence Read Archive (SRA) database, we found one of the two sequenced genes did not express in any of the maize tissues analyzed and the other one expressed like a housekeeping gene. The third one was specifically expressed in the anther. Further analysis of this candidate gene and fine mapping are still in progress.

P51

Deciphering the potential tolerance mechanisms against waterlogging stress at maize seedling stage through physiological study and proteomic technique

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Waterlogging has long been identified as a major abiotic stress caused by poor drainage, flooding and long periods of rain. It always hampers plant growth and results in dramatic losses in crop production. To understand the molecular mechanisms of waterlogging tolerance, proteins from the roots of maize lines A3237 (tolerant) and A3239 (sensitive) were extracted under normal and waterlogged conditions at 3 d after treatment and analyzed by iTRAQ LC-MS/MS technique. There were 324 proteins identified as significantly responding to waterlogging stress, 228 proteins from A3237 and 178 from A3239; among them, 82 proteins had the same expression patterns both in A3237 and A3239. The majority of the proteins responding to waterlogging were involved in metabolism, transporters, energy, disease/defense and signal transduction for both A3237 and A3239. Furthermore, physiological assays revealed that high activity of SOD and CAT and high content of GSH in A3237 could produce low contents of H2O2 and MDA, and it was consistent with the high abundance proteins related to disease/defense in A3237. Our data indicated that maize roots can resist waterlogging via multiple mechanisms. Perceiving the waterlogging stress, plants altered their metabolic pattern including primary and secondary metabolism – decreased energy consumption and increased ATP production, sustained pH stability, enhanced signal transduction, improved antioxidative ability and increased expression of cell wall degradation proteins to suppress primary root elongating growth. All these reactions comprised a complex network to prolong survival. These results provide new insights into uncovering tolerance mechanism of waterlogging stress at the maize seedling stage at the proteomic level.

P52

Defining the SUMOylation System in *Zea mays* and its Roles in Stress Protection

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Plants rapidly initiate a variety of cellular responses to cope with environmental challenges. Among the fastest is the conjugation of small ubiquitin-related modifier (SUMO) to an array of mostly nuclear target proteins via a pathway that is essential for optimal stress tolerance. Despite the importance of SUMOylation to stress protection, little is know about this modification in crop species. Here, *in silico* approaches were used to identify all major SUMO pathway components in maize (*Zea mays*). This list includes: three SUMO genes, two of which encode identical proteins related to *Arabidopsis SUMO1*, and a third more divergent SUMO isoform, E1, E2, and E3 enzymes involved in conjugation, and an array of deSUMOylating proteases that reverse the modification. Phylogenetic analyses reveal that most plants have a non-conserved SUMO gene along with at least one canonical SUMO gene similar to *Arabidopsis SUMO1*. The split suggests an ancient duplication event with canonical SUMOs maintaining an essential role and non-canonical forms acquiring divergent functions. Additional SUMO-encoding genes include a monocot-specific *DiSUMO-like* gene bearing two SUMO β-grasp folds in tandem and a conserved *SUMO variant* gene with an elongated, charged N-terminal half followed by the β-grasp fold. The E2 gene family has six members with a subset appearing to have subfunctionalized based on phylogenetic and tissue-specific expression analyses. A novel, conserved, plant-specific E3 ligase bearing a signature MIZ/SP-RING domain was discovered along with the E3 genes *SIZ1* and *MMS21*. *In vitro* assays using recombinant E1 and E2 enzymes demonstrate the functionality of the maize SUMO machinery. Like *Arabidopsis*, maize rapidly SUMOylates an array of proteins *in planta* after heat, salt, and oxidative stress. We have generated transgenic germplasm to define the maize SUMOylome by proteomic approaches. Collectively, these studies define the organization of the maize SUMO system and provide a springboard for deeper functional characterizations.

Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF)

P53

Develop a multi-parent advanced generation inter-cross population for complex quantitative traits dissection in maize

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To investigate the genetic architecture of complex quantitative traits, we introduce a modified multi-parent advanced generation inter-cross (MAGIC) population in maize which derived from 24 elite Chinese maize inbred lines from "Huangzao 4" heterotic group and consisted of 1664 progenies. The twenty-four founders were crossed using NCII design to create 140 F1s in summer 2004. Thirty F1s which had better heterotic performance were selected to cross using NCII design, and the rest were open pollinated in the isolated region to create four-way F1s in winter 2004. The seeds were mixed together with the ratio of 2:1 to improve the lines per se performance of the population. Then the four-way F1s were open pollinated from summer 2005 to winter 2008 in the isolated region, up to 8 generations. From summer 2009, the lines were self-pollinated continuously for 6 generations and obtained 1664 inbred lines.
Preliminary filed test were performed in 2013 in three locations, huge phenotypic variations were observed for all the measured traits. Broad sense heritability of the agronomic traits ranged between 0.59 for leaf number below ear and 0.83 for plant height. Two elite testers were chosen based on the heterotic performance and breeding practice in China to cross with all the inbred lines of MAGIC population. In the summer 2014, we will phenotype all the MAGIC lines per se and the tester hybrids in multiple environments. Meantime, all the lines will be genotyped using genotyping-by-sequencing technique. Using this population, we expect to fine map and clone major QTLs to dissect the genetic basis of the complex quantitative traits, study heterosis of MAGIC population, and provide useful resources for maize breeding.

Funding acknowledgement: This research was supported by the National Hi-Tech Research and Development Program of China and the National Natural Science Foundation of China

P54

Developmental processes controlling seed size in maize evaluated in the Krug seed size populations and derived inbreds

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Seed size is an important yield component in maize, having a role in crop domestication and artificial selection. Three seed components of maize, embryo, endosperm and pericarp, which possess different complements of maternal and paternal genomes, undergo coordinated growth through cell division and expansion to determine the final size of the seed. Comprehensive understanding of the molecular and genetic regulation of seed size is crucial both for broadening the knowledge of seed development and for reaching maximum seed yield in crop plants. To achieve this goal, two maize populations, the Krug Large Seed (KLS30) and the Krug Small Seed (KSS30) populations recurrently selected for 30 cycles for divergent seed size were analyzed. Our previous research showed that KLS30 has larger and heavier seeds than KSS30. This is due to a faster rate of grain fill and a slower progression through development in KLS30 than KSS30. In this study, inbred lines derived from KLS30 and KSS30 were self- and cross-pollinated to uncover new anatomical aspects governing seed size and to characterize maternal contributions controlling the rate of seed development. Imaging of developing kernel sections by microscopy showed that the large seed size of KLS30 inbreds is more attributed to cell number than cell size. Dry weight accumulation of developing seeds at three day intervals across seven stages showed a faster progression through development in KSS30 compared to KLS30 inbreds. Analysis of endoreduplication and transcription will provide additional insight into the process of seed development in these dramatically phenotypically diverse populations and lines.

Funding acknowledgement: National Science Foundation (NSF), China Scholarship Council (CSC)

P55

Different Expression Analysis of NADH Dehydrogenase in Maize CMS-C

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The mechanisms of abortion and fertility restoration in maize CMS-C remains ambiguous.In the study,NADH-ubiquinone oxidoreductase B17.2 (39% sequence coverage)and 51 kDa subunit(42% sequence coverage) detected by 2-D electrophoresis were absent in C48-2. NHADK gene(69.01% gene coverage) was down-regulated in C48-2 compared with N48-2 by RNA-Seq. Furthermore, a novel transcript of nad4 coding NADH dehydrogenase 4 subunit was found in C48-2 based on RNA-Seq. Amusing, these differential expressed genes or proteins all involved in the complex I (NADH-ubiquinone oxidoreductase), the expression pattern from proteins level were consistent with that from RNA level. These results indicate that complex I may play a key role in the anther development.

P56

Different Response to Lead-Stress of line 178、9782 and the F1 generation of 178 and 9782

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Lead (Pb) as one of the most abundant heavy metal has threatened the environment. But the heavy metals tolerance mechanism of plants is not clear now. In this study, Pb-highly resistance maize line 178, Pb-sensitive maize line 9782 and F1 were chosen to analyze the different response to Pb-stress to find some Pb resistance related genes or Pb resistance mechanism. 178's root growth traits under Pb stress were weaker than 9782 and F1, but the root relative activity (to CK) of 178 and F1 were both higher than 9782. The MDA concentration of 178 and F1 were both lower than 9782 under Pb-stress. After treated by Pb, only weak cell wall was found of the 178 root surface, but weak cell wall and something like neoplasms were both discovered under SEM. The ROS protective enzyme relative activities of 178 were higher than 9782 and F1. After qRT-PCR, we find 11 different expression genes in 178 and 9782. The aquaporin PIP1-6 expression down regulated in 178, but up/down-regulated alternately in 9782. The expression of MADS-box genes of 178 up regulated more than 9782. The Glutathione S-transferases expression level up-regulation folds of 178 was higher than 9782, but regulation number times was less than 9782. The GRMZM2G093286 expression of 178 and 9782 up regulated in previous 2H but down regulated in the follwing treatment time. The expression up-regulation folds of GRMZM2G162758, Cysteine proteases and 30S ribosomal protein S15 of 178 was higher than 9782. The beta-fructofuranosidase expression was induced by Pb in 178, but inhibited in most treat time. AKINbetagamma-1 protein kinase was inhibited in previous 2H treatment time but was induced, but 9782 up regulated the expression level in previous 12 H and down-regulated in the other time.The GRMZM2G115190 expression of 178 up/down-regulated alternately, but was inhibited in 9782 all the time. These differential expression genes may play an emportant role in Pb-stress resistance of maize line.

Funding acknowledgement: National Natural Science Foundation of China

P57
DNA elements required for the *Bx*-gene expression

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Benzoxazinoids are constitutive defence related secondary metabolites that are found in many grasses, including maize, wheat and rye. The major benzoxazinoid in maize is DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one). DIMBOA-biosynthesis has been elucidated in maize. A gene cluster comprising the biosynthetic genes (*Bx1* to *Bx8*) maps on short arm of chromosome 4. *Bx1* is the branchpoint gene of benzoxazinoid biosynthesis.

The inbred lines B73 and Mo17 differ with respect to DIMBOA content in older plantlets (24 days after imbibition). Using the recombinant inbred line population IBM (intermated B73 x Mo17), a major QTL (Quantitative Trait Locus) for high late DIMBOA levels was identified on chromosome 4, close to the *Bx* -gene cluster. The maize inbred line Mo17 is unique in the diversity panel of 25 inbred lines (NAM founder lines) with respect of high late *Bx1* expression. Analysis of the hybrid progeny between Mo17 and B73 revealed that the Mo17 allele of *Bx1* is almost exclusively expressed at later stage, and, the *Bx1* expression rate is significantly higher in the hybrid than in B73.

Fine mapping of the chromosome 4 *QTL* uncovered a duplicated sequence (*αβ*-duplicate), located 151 kb upstream of *Bx1* that is present in the NAM founder lines exclusively in Mo17. This sequence element is required for high and allele-specific late *Bx1* expression. Interestingly, the*αβ*-duplicate is located in a hotspot of recombination. Further element(s) required for high and allele-specific expression could be localized between the *αβ*-duplicate and the *Bx1*-gene. Analysis of hybrids, recombinant lines and near isogenic lines (NILs) revealed that late *Bx1*expression is also negatively influenced by a *trans*-factor contributed by B73. This factor is not found in proximity to the gene cluster.

Funding acknowledgement: DFG SFB924 project

P58
Embryo lethal plastid translation mutants and their genetic suppressors in maize

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In plants, mutations that disrupt plastid translation typically cause embryo lethal phenotypes. In maize seed, such mutations severely perturb embryo development, but have little impact on endosperm development, indicating that plastid translation is essential for embryo development but not for endosperm formation. A mutant in plastidial Ribosomal Binding Protein A (*rbpA*) gene was identified in UniformMu W22 inbred transposon population as an early embryo lethal mutant. The gene is required for the processing of plastidial 16S rRNA in 30S ribosome biogenesis in Arabidopsis. When the *rbpa* mutant was crossed to the B73 inbred and subsequently self-pollinated, the embryo lethal phenotype was suppressed in F2 seeds, conditioning viable embryos that germinate to produce albino seedlings. This finding adds to growing evidence supporting the hypothesis that the requirement of plastid translation for embryogenesis can be bypassed in certain genetic backgrounds. To further test the hypothesis that background suppressors specifically modify plastid translation mutants, we isolated additional embryo lethal mutants from the UniformMu transposon population that include genes involved in plastid translation as well as essential genes that have non-plastid functions. We have crossed these mutants with B73 inbred and generated F2 seeds to determine whether the embryo lethality was also suppressed in all embryo lethal mutants or only plastid translation-related mutants. Our genetic and molecular study of embryo lethal suppressors will provide insight into the specific role of plastids in embryogenesis.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P59

Expanding the toolbox to study the dynamics of molecular and cellular processes in the maize leaf growth zone

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One of the most fascinating open questions in biology is how organ and organism size is controlled. The maize leaf offers an excellent experimental system to study leaf growth, due to the linear organization of cell division and expansion along its longitudinal axis: active cell divisions occur at the base of the leaf, and as the distance from the base increases cells will cease division and start expanding until they reach their mature cell size. Recently, we identified a cellular and molecular mechanism that characterizes the transition from cell division to expansion. We found that bioactive gibberellins (GAs) peaked near the transition, and we genetically showed the functional importance of the position of this transition zone for final organ size.
The aim of our research is to map the dynamics of cellular and molecular processes during the transition between cell division and cell expansion with high resolution throughout the growing maize leaf. In order to improve our knowledge of the growth processes at the cellular level, we are correlating growth processes to physiological and cell-type specific changes in the growth zone and we are constructing a 3D cellular map of the growth zone. These phenotypic data will be further analyzed in relation to changes in the cell-type specific transcriptome sampled using laser micro-dissection combined with RNAseq. In addition, we introduced interactomics tools, such as tandem affinity purification, in maize to study the changes in sub-unit composition of protein complexes in the leaf and ear growth zone. We will use in house developed tools, such as Cornet and PLAZA to computationally analyze and to integrate and visualize the data. Finally, an automated phenotyping platform, called Phenovision, will be used to functionally characterize putative regulatory genes.

P60

Fine Mapping and Cloning the Unidirectional Cross Incompatibility gene *Ga1-m* in Maize (*Zea mays* L.)

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The unidirectional cross incompatibility genes exist in teosinte and many popcorns. A Chinese popcorn inbred line SDGa25 carries the unidirectional cross incompatibility gene *Ga1* (gametophyte 1) and the *Ga1-m* was mapped between markers SD3 and SD12 on maize chromosome 4. The physical distance was about 2M bp based on the *B73 RefGen_v2 sequence*. In this study, *Ga1-m* was fine mapped using the new developed markers between SD3 and SD12. The *Ga1-m* gene was mapped between SNP markers 13-4 and 25-5. The physical distance was about 246Kbp based on the *B73 RefGen_v2 sequence*. In the mapping region, there are three interesting candidate genes GRMZM2G419836, GRMZM2G027021 and GRMZM2G039983. GRMZM2G419836 is a member of the thioredoxin superfamily. GRMZM2G027021 is a GTP-binding protein which is involved in pollen tube growth. GRMZM2G039983 has homology to WDL1 of Arabidopsis which is a microtubule-associated protein. All the three genes may be involved in pollen-pistil interactions. In order to isolate the *Ga1-m* gene, the SDG25 BAC library was constructed and screened. The primers used in screening the BAC library were primers of fine mapping. The positive recombinant BAC clones covering the whole mapping interval were identified and being sequenced.

P61

Fine mapping of leafy, a dominant mutant conferring extra leaves above ear in maize

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Leafy (lfy1) is a dominant mutant in maize reported by DonaId L.Shaver in 1983. It delays flowering time and produces more leaves, specifically above the ear. Due to the more extra leaves phenotype, it has been used as a very important germplasm to increase leaf biomass in maize breeding programs especially for silage production. The lfy1 locus was reported to be located on the long arm of Chromosome 3 and had not been molecularly isolated. In this study, lfy1 mutant was found not completely dominant according to three F2 populations derived from lfy1 mutant. In the cross of Lfy1×B73, the F2 population showed a distribution of 3:1, indicating that lfy1 was controlled by a single dominant gene. Later, 4820 non-leafy individuals from this population were used to fine mapping lfy1 gene with newly developed makers. Lfy1 gene was assigned to a 90kb interval flanked by maker Indel072028 and Indel458095. Only two genes, GRMZM2G072052 and GRMZM2G072080, were in this region based on the B73 reference sequence. The polymorphism makers on the two candidate genes were all co-segregation with leafy phenotype and helped us to observe the abnormal phenomena on the shoot apical meristem of lfy1 mutant in seedling stage. Our study will contribute to the further isolation of lfy1 gene and advance our understanding of lfy1 gene function.

P62

Functional divergence of the paralogous LATERAL ORGAN BOUNDARIES DOMAIN proteins RTCS and RTCL during shoot-borne root development in maize (*Zea Mays* L.)

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Maize is characterized by a complex root system consisting of the embryonic primary root and seminal roots, and an extensive postembryonic, shoot-borne root system. Shoot-borne crown and brace roots make up the major backbone of the adult root system. The *rtcs* (*rootless concerning crown and seminal roots*) mutant is defective in seminal and shoot-borne root initiation. The *rtcs* gene encodes a member of the plant-specific LBD (Lateral Organ Boundaries Domain) protein family. Due to an ancient maize whole-genome duplication, the paralogous *rtcs* and *rtcl* (*rtcs-like*) genes that are highly conserved in their LOB domains were generated. Both genes are auxin inducible and display conserved molecular interactions. A novel knockdown mutant of *rtcl* containing a 46 bp deletion upstream of the ATG start codon of *rtcl* was isolated. In contrast to *rtcs*, the *rtcl* mutant displays normal root initiation but impaired shoot-borne crown root elongation. Although the total number of crown roots was not affected, fewer crown roots that are longer than 5 cm were detected in 10-day-old *rtcl* mutant seedlings compared to the wild-type plants. In addition to the lack of seminal and shoot-borne root initiation already observed in the *rtcs* single mutant, the *rtcs/rtcl* double mutant does not display any novel aberrant root phenotypes such as lateral root density and primary root elongation, indicating a specific function of*rtcl* during crown root development. Moreover, qPCR analyses revealed that *rtcl* expression is repressed by *rtcs* but *rtcs* is regulated independently by *rtcl*. Taken together, the paralogous LATERAL ORGAN BOUNDARIES DOMAIN proteins RTCS and RTCL display functional divergence during shoot-borne root development in maize.

P63

Genome Expression Profile Analysis of the Immature Maize Embryo during Dedifferentiation

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Maize is one of the most important cereal crops worldwide and one of the primary targets of genetic manipulation, which provides an excellent way to promote its production. However, the obvious difference of the dedifferentiation frequency of immature maize embryo among various genotypes indicates that its genetic transformation is dependence on genotype and immature embryo-derived undifferentiated cells. To identify important genes and metabolic pathways involved in forming of embryo-derived embryonic calli, in this study, DGE (differential gene expression) analysis was performed on stages I, II, and III of maize inbred line 18-599R and corresponding control during the process of immature embryo dedifferentiation. A total of ,21 million cDNA tags were sequenced, and 4,849,453, 5,076,030, 4,931,339, and 5,130,573 clean tags were obtained in the libraries of the samples and the control, respectively. In comparison with the control, 251,324 and 313 differentially expressed genes (DEGs) were identified in the three stages with more than five folds, respectively.Interestingly, it is revealed that all the DEGs are related to metabolism, cellular process, and signaling and information storage and processing functions. Particularly, the genes involved in amino acid and carbohydrate transport and metabolism, cell wall/membrane/envelope biogenesis and signal transduction mechanism have been significantly changed during the dedifferentiation. To our best knowledge, this study is the first genome-wide effort to investigate the transcriptional changes in dedifferentiation immature maize embryos and the identified DEGs can serve as a basis for further functional characterization.

Funding acknowledgement: Ministry of Agriculture of the People's Republic of China

P64

Genome expression profile analysis reveals important transcripts in maize roots responding to the stress of heavy metal Pb

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Lead (Pb) has become one of the most abundant heavy metal pollutants of the environment. With its large biomass, maize could be an important object for studying the phytoremediation of Pb-contaminated soil. In our previous research, we screened 19 inbred lines of maize for Pb concentration, and line 178 was identified to be a hyperaccumulator for Pb in both the roots and aboveground parts. To identify important genes and metabolic pathways related to Pb accumulation and tolerance, line 178 was underwent genome expression profile under Pb stress and a control (CK). A total of ~11 million cDNA tags were sequenced and 4,665,539 and 4,936,038 clean tags were obtained from the libraries of the test and CK, respectively. In comparison to CK, 2379 and 1832 genes were identified up- or down-regulated, respectively, more than five folds under Pb stress. Interestingly, all the genes were related to cellular processes and signaling, information storage and processing, or metabolism functions. Particularly, the genes involved in posttranslational modification, protein turnover, and chaperones; signal transduction, carbohydrate transport and metabolism; and lipid transport and metabolism significantly changed under the treatment. In addition, seven pathways including ribosome, photosynthesis, and carbon fixation were affected significantly, with 118, 12, 34, 21, 18, 72 and 43 differentially expressed genes involved. The significant up-regulation of the ribosome pathway may reveal an important secret for Pb tolerance of line 178. And the sharp increase of laccase transcripts and metal ion transporters were suggested to account in part for Pb hyperaccumulation in the line.

Funding acknowledgement: National Natural Science Foundation of China

P65

Genome-wide association study dissects the genetic architecture of carotenoid biosynthesis in maize kernels

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Carotenoids are natural pigments which play an important role in human health. To dissect the genetic basis of maize carotenoid biosynthesis and accumulation, a genome-wide association study was performed using 1.06 million SNPs and seven carotenoid related traits in a maize panel of 508 maize inbred lines. We identified 18 loci significantly associated with carotenoid related traits in maize kernels at $P < 1.8 \times 10^{-6}$, which we further validated using linkage mapping and expression quantitative trait loci (eQTL) mapping. Over half of the detected loci had the evidence support of QTL (11/18) and eQTL (13/18). The later demonstrates that transcript regulation may be a major molecular mechanism in the regulation of the natural variations of carotenoid biosynthesis and accumulation. These results provide useful insight into the genetic basis of carotenoid biosynthesis in maize kernels and biofortification of maize carotenoid related traits.

Funding acknowledgement: National Natural Science Foundation of China

P66

Genome-wide identification of miRNAs and their targets in response to low-nitrogen stress in maize

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MicroRNAs (miRNAs) play an important role in response and adaptation of plants to many stresses including LN. Characterizing relevant miRNAs will improve our understanding of NUE and LN tolerance, thus contributing to sustainable maize production. Our objective was to identify novel and known miRNAs and their targets involved in NUE and LN tolerance in maize (Zea mays L.) . MiRNAs were identified by deep sequencing and the microarray systems and their targets were analyzed together through deep-sequencing the degradome libraries. The identity of target genes was confirmed by gene-specific RNA ligase mediated 5′ rapid amplification of cDNA ends (RLM-RACE) and quantitative expression analysis. Over 150 million raw reads of small RNA and degradome sequence data were generated. A total of 46 unique mature miRNA sequences belonging to 23 maize miRNA families were sequenced. Eighty-five potentially new miRNAs were identified, with corresponding miRNA* also identified for 65 of them. Twenty-five new miRNAs showed over 2-fold relative change in response to LN. Nine miRNA families (miR164, miR169, miR172, miR397, miR398, miR399, miR408, miR528, and miR827) were identified in leaves, while nine others (miR160, miR167, miR168, miR169, miR319, miR395, miR399, miR408, and miR528) identified in roots. In addition to known miR169 species, two novel putative miR169 species were identified. Deep sequencing of miRNAs and degradome and RLM-RACE and quantitative PCR analyses of their targets showed that miRC10- and miRC68-mediated target cleavage may play a major role among miR169 families in the adaptation of maize seedlings to LN . Small RNA and degradome sequencing combined with qRT-PCR and RLM-RACE verification enabled the efficient identification of miRNAs and their target genes. The generated data sets and the knowledge gained will help understand the roles of miRNAs in maize responding to nitrogen limiting environments and eventually develop strategies for maize improvement.

Funding acknowledgement: National Science Foundation of China, China Ministry of Science and Technology

P67

Genome-wide identification, splicing and expression analysis of the myosin gene family in maize (*Zea mays*.)

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The actin-based myosin system is essential for the organization and dynamics of the endomembrane system and transport network in plant cells. Plants harbor two unique myosin groups, class VIII and class XI, and the latter is structurally and functionally analogous to the animal and fungal class V myosin. Little is known about myosins in grass, even though grass includes several agronomically important cereal crops. Here, we identified 14 myosin genes from the genome of maize (*Zea mays*). The relatively larger sizes of maize myosin genes are due to their much longer introns, which are abundant in transposable elements (TEs). Phylogenetic analysis indicated that maize myosin genes could be classified into class VIII and class XI, with three and eleven members, respectively. Apart from subgroup XI-F, the remaining subgroups were duplicated at least in one analyzed lineage, and the duplication events occurred more extensively in Arabidopsis than in maize. Only two pairs of maize myosins were generated from segmental duplication. Expression analysis revealed that most maize myosin genes were universally expressed, whereas a few members (XI-1, 6 and 11) showed an anther-specific pattern, and many underwent extensive alternative splicing. We also found a short transcript at the O1 locus, which conceptually encoded a headless myosin that most likely functioned at the transcriptional level rather than via a dominant-negative mechanism at the translational level. Together, these data provide significant insights into the evolutionary and functional characterization of maize myosin genes that could transfer to the identification and application of homologous myosins of other grasses.

Funding acknowledgement: National Natural Sciences Foundation of China (31370035, 31000747 and 31171559), Ministry of Science and Technology of China (2012AA10A305 and 2014CB138204)

P68

Genome-wide transcriptional profile analysis of Al-responsive genes in maize

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Aluminum (Al) toxicity is a major factor limiting crops production on acid soils, which comprise up to 50% of the world's arable land. To extend the understanding of the molecular mechanisms of maize Al tolerance, microarray technology was used to analyze the gene expression profiles in maize inbred line 178 under Al stress. Among 2,487 significant changed genes, 634 genes were up-regulated and 330 genes were down-regulated at least twofold when exposed to 60 μmol Al3+. Up-regulated genes were related to cell-wall modification, abiotic and biotic stress responses as well as signal transduction, while down-regulated genes were involved in primary metabolism, secondary metabolism, protein synthesis and processing, and cell cycling. Interestingly, genes that encoding enzymes of TCA cycle were not significantly changed at the transcript level, suggesting that synthesis of organic anions in response to Al may not be transcriptional regulated. Despite some of previously reported Al-responsive genes, multiple members of the WRKY transcriptional family were also identified up- or down-regulated by Al stress in our microarray data. The genome-wide transcriptional gene express profiles may favor the understanding on the molecular basis of Al toxicity and Al tolerance in maize.

Funding acknowledgement: National Science Foundation (NSF)

P69

Identification and characterization of an E3 ubiquitin ligase Rbx1 in maize (Zea mays L.)

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E3 ubiquitin ligases catalyze the ubiquitination of a variety of biologically significant protein substrates for targeted degradation through the 26S proteasome, as well as for nonproteolytic regulation of their functions or subcellular localizations. Here we report the identification and characterization of an E3 ubiquitin ligase, the Ring box1 (Rbx1) homologue in maize, which is designated as Zm-Rbx1. Analysis of the genomic organization showed that the gene of Zm-Rbx1 belonged to the chromosome 4 of maize and contained five exons and six introns. Amino acids sequence analysis revealed that Zm-Rbx1 contained conserved cysteine/histidine residues, which are the characteristics of Rbx proteins. Real-time PCR analysis revealed that the expression levels of Zm-Rbx1 increased quickly after salicylic acid (SA), jasmonic acid (JA) and sugarcane mosaic virus (SCMV) challenge. Then we suggest that Zm-Rbx1 is involved in the defense response of maize, although detailed molecular mechanism needs to be further studied. After prokaryotic expression and purification of the recombinant Zm-Rbx1 protein from E. coli BL21 (DE3) cells, the ubiquitination assay demonstrated that Zm-Rbx1 showed ubiquitin ligase activity.

Funding acknowledgement: National Natural Science Foundation of China

P70

Identification and characterization of the Zinc-regulated transporters, Iron-regulated transporter-like Protein (ZIP) gene family in maize

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The Zinc-regulated transporters, Iron-regulated transporter-like Proteins (ZIP) are capable of uptaking and transporting divalent metal ion and are suggested to play critical roles in balancing metal uptake and homeostasis, though a detailed analysis of ZIP gene family in maize is still lacking. Nine ZIP-coding genes were identified in maize genome. It was revealed that the ZmZIP proteins share a conserved transmembrane domain and a variable region between TM-3 and TM-4. Transiently expression in onion epidermal cells revealed that all ZmZIP proteins were localized to the endoplasmic reticulum and plasma membrane. The yeast complementation analysis was performed to test the Zn or Fe transporter activity of ZmZIP proteins. Expression analysis showed that the ZmIRT1 transcripts were dramatically induced in response to Zn- and Fe-deficiency, though the expression profiles of other ZmZIP changed variously. The expression patterns of ZmZIP genes were observed in different stages of embryo and endosperm development. The accumulations of ZmIRT1 and ZmZIP6 were increased in the late developmental stages of embryo, while ZmZIP4 was up-regulated during the early development of embryo. In addition, the expression of ZmZIP5 was dramatically induced associated with middle stage development of embryo and endosperm. These results suggest that ZmZIP genes encode functional Zn or Fe transporters that may be responsible for the uptake, translocation, detoxification and storage of divalent metal ion in plant cells. The various expression patterns of ZmZIP genes in embryo and endosperm indicates that they may be essential for ion translocation and storage during differential stages of embryo and endosperm development. The present study provides new insights into the evolutionary relationship and putative functional divergence of the ZmZIP gene family during the growth and development of maize.

Funding acknowledgement: National Special Program for GMO Development of China (grant number 2008ZX08003-002).

P71

Identification and functional analysis of differentially expressed miRNA in maize (Zea mays L.) in response to Banded Leaf and Sheath Blight

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MicroRNAs (miRNAs) are a class of small non-coding RNAs that negatively regulate special target mRNAs at the post-transcriptional level by directing target mRNA cleavage or translational inhibition. Plant miRNAs have been implicated in developmental processes and adaptations to environmental stresses including biotic- and abiotic-stresses. The maize banded leaf and sheath blight (BLSB) is a bio-stress that is highly harmful to plants. To investigate the responsive functions of miRNAs under BLSB stress, miRNA expression in BLSB-stressed Maize (Zea mays L.) was profiled using Deep sequencing. A total of 41 known miRNAs and 39 novel BLSB-responsive miRNAs were identified, of which 9 were further validated experimentally and two important miRNA candidates were analyzed by ISH. Target genes were also predicted for these BLSB-responsive miRNAs, which encoded transcription factors, and proteins associated with metabolic processes or stress responses. In addition, the mRNA levels of several targets were negatively correlated with the corresponding miRNAs under BLSB stress. These findings suggested that miRNAs has played an important role in BLSB tolerance in maize and highlighted a novel molecular mechanism of BLSB tolerance in plants.

Funding acknowledgement: National Hi-Tech program

P72

Identification of candidates for two major QTLs that confer resistance to gray leaf spot in maize

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Gray leaf spot (GLS), caused by the causal fungal pathogen *Cercospora zeae-maydis* and *Cercospora zeina*, is one of the most serious foliar diseases of maize worldwide. In our current study, a highly resistant inbred line Y32 and a susceptible line Q11 were used to produce segregating populations for QTL mapping. In initial QTL analysis, four QTLs, located on chromosomes 1, 2, 5, and 8, were detected to confer GLS resistance. Each QTL could explain 2.53 to 23.90% of the total phenotypic variation, predominantly due to additive genetic effects. Two major QTLs, *qRgls1* and *qRgls2*, consistently detected across different locations were located on bins 8.01/03 and 5.03/04, and could steadily increase the resistance percentages by 20 to 61% and 15 to 29%, respectively. With a recombinant-derived progeny test strategy, we narrowed down *qRgls1* from an initial ~23Mb to ~800kb intervals and *qRgls2* from ~110Mb to ~1Mb, respectively. We then screened the resistant Y32 BAC library to yield a number of positive clones. The minimal tilling Y32 BAC clones were subjected to sequencing and gene annotation. Comparison of the resistant Y32 and susceptible B73 for the predicted genes enable us to identify the candidates for both *qRgls1* and *qRgls2*. A cluster of five kinase genes are most likely to be candidates for *qRgls1*; while, another kinase gene was speculated to be the candidate for *qRgls2*.

Funding acknowledgement: The national ‘863’ high-tech program of China

P73

Identification of QTL for Starch, protein and Oil content in maize kernel at different filling stages under multi-environments

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In maize kernels starch, protein and oil are important nutrition components. A population of 166 recombinant inbred lines derived from a maize hybrid Nongda108(Huang-C×Xu178) was used to analysis the genetic background among starch, protein and oil content at different filling under multi-environments by unconditional and conditional QTL mapping. Combined phenotypic data during five filling stages in three sites with a genetic linkage map constructed using 203 markers, 12, 11, and 13 unconditional QTL were detected for starch, protein and oil content. In conditional QTL mapping, eight, seventeen and sixteen conditional QTL for starch, protein and oil content were discovered. Some QTL for starch, protein and oil content were clustered in the same genomic region. Several QTL for starch, protein and oil content were detected over two stages or sites. One, two and two QTL for starch, protein and oil content were identified under unconditional and conditional QTL mapping. The Results showed that the genetic mechanisms of starch, protein and oil content were very complicated, but there is a strong genetic correlation among the three key nutrition components.

Funding acknowledgement: National Natural Science Fondation of China

P74

Identification the reference genes of maize and tesing by qRT-PCR

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In maize, there has no systematic analysis to determine a number of candidates and identify the best suitable reference genes. Using the public microarray data to select and identity the novel reference genes for maize. In this species, HKGS is used to normalize the qRT-PCR data, but the HKGS is not stability expression in various experimental conditions and tissues. So the novel reference genes were selected from microarray and identified by qRT-PCR and normalized by normalization software. The results showed that the novel reference gene had more stability than HKGS. Therefore, the novel reference genes have a extensive application in maize normalization.

P75

Improvement of amylose synthesis in maize endosperm through RNAi down-regulating starch branching enzyme IIa and IIb

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Corn starch play an important role in industries and food production, which consist of linear amylose (<30%) and highly branched amylopectin (>70%). Amylose receives extensive industrial interest and many efforts have been made to elevate amylose content in maize endosperm. The synthesis of amylose in maize endosperm is controlled by several genes and in which starch branching enzyme IIb (SBEIIb) plays the most important roles on amylase content. However, conventional breeding material from *sbe2b* mutant showed severe grain yield and starch reduction. In this work, we use RNAi technology to suppress the expression of SBEII genes and ten hairpin *SBEII*RNAi vectors were constructed and transformed maize inbred line Chang 7-2, targeting a conserved domain of *SBEIIa* and *SBEIIb* with 893nt or 467nt, or both a *SBEIIa* specific area with 415nt or 154nt, and a 295nt of *SBEIIb* specific area, which were driven by the constitutive CaMV 35S promoter (P35S) or endosperm-specific 27kD zein promoter (P27kD), respectively. These*SBEII*RNAi transgene led to down-regulated SBEII expressions and SBE activity, enhanced amylose content with varying degrees, and slightly decreased total starch content in endosperm and yield compared with WT. The results showed that the RNA interference constructs driven by P27kD were more efficient than that by P35S; the hpRNA construct targeting both *SBEIIa* and *SBEIIb* specific area gave the most obvious silencing efficacy; and the inclusion of catalase intron in *SBEII*RNAi constructs had better interference effect than the chalcone synthase intron. In terms of the SBEII expression levels and amylose content, we confirmed the most efficient *SBEII*RNAi construct, which targeted both a 415nt of *SBEIIa* specific region and a 295nt *SBEIIb* specific region driven by P27kD, and the corresponding transgenic lines showed amylose content over 55%. Amylose and starch content of the homozygous transgenic lines showed little alteration of generations, indicating that the *SBEII*RNAi transgene could stably inherit in maize endosperm. This work has obtained a new high amylose maize material by using RNAi technology, and accumulated important data for high amylose corn creation through transgenic technology.

Funding acknowledgement: National Program of Transgenic Variety Development of China

P76

Introgression of QPM trait into non-QPM elite maize inbred lines through marker-assisted breeding

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The low nutritive value of normal maize, due to deficiency of two nutritionally vital amino acids, lysine and tryptophan is genetically corrected in biofortified form known as Quality Protein Maize (QPM).QPM breeding can alter the protein composition of the maize endosperm resulting in twice the amount of lysine and tryptophan. Marker assisted selection (MAS) in combination with conventional breeding can greatly accelerate the introgression of opaque2 (o2) genotype into normal maize for the development of high quality protein versions. Normal inbred of promising medium maturity hybrid, EHL 161708 was targeted for conversion using simple sequence repeats (SSR) markers viz., phi057 and umc1066. The cross was attempted between BAJIM-08-27 (non-QPM) and CML-193 (QPM donors) to get heterozygous plants (F1). Foreground selection of the F1's was done using phi057 and umc1066 SSR markers. The selected heterozygotes were backcrossed with their respective recipient parent to generate backcrossed population. A total of 20 SSR markers of chromosome 7 were surveyed on BC2F1 population to find flanking markers for o2 locus. The flanking markers (mmc0171 and umc2325) were used for the selection of single and double recombinants. The selected single recombinants were subjected to whole genome background selection in BC2F1 generation and identified 85-90% recurrent parent genome recovery in few plants.The plants showing 90% recurrent parent genome recovery were selfed to generate BC2F2 generation and were surveyed for homozygous o2 locus and then selfed to get BC2F3 generation. The BC2F3 seeds were phenotypically evaluated for kernel modification and tryptophan concentration. Kernels with less than 25% opaqueness were selected over 25–50% and more than 50% opaqueness. The tryptophan concentration in endosperm protein was significantly enhanced as compared to original recipient line.

Funding acknowledgement: Department of Biotechnology, New Delhi, India

P77

Isolation and functional analysis of ZmPHR genes in maize

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Phosphorus (P) is an essential macronutrient required for plants growth and development. Although P is abundant in soil, the concentration of plant-available phosphate (Pi) is often low. Previous research has demonstrated that PHR1 plays a central role in Pi-starvation signaling in Arabidopsis thaliana. The function of PHR1-like gene in maize, however, has not been studied. In this work, five PHR1-like genes named ZmPHR1 through ZmPHR5 were isolated from the genome of maize using the AtPHR1 protein sequence as a query. Subcellular localization predicted that they all localize in the nucleus. Phylogenetic analysis indicated that they all belong to the same subfamily of MYB-CC transcription factor as AtPHR1, OsPHR1 and OsPHR2. Each of the genes was constructed to an over-expression vector and then transformed to wild type Arabidopsis plants, respectively. A series of homozygous transgenic lines which over-express each of the genes were obtained. For comparison with the wild type plants, the transgenic lines will be investigated for growth and development, anthocyanins accumulation, contents of Pi between roots and shoots, expression profiles of a subset of Pi starvation-inducible genes, under both Pi- sufficient (1250μM) and Pi-starvation (10μM) conditions. The function of ZmPHR genes in regulation of the Pi-starvation signaling pathways in maize will be summarized.

P78

Maize NCP1 is an EAR motif-lacking NINJA family protein that negatively regulates drought and ABA responses through interacting with and inhibiting the activity of transcription factor ABP9

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ABA signaling plays pivotal roles in plant responses to abiotic stress. Maize ABP9 is an ABRE-binding bZIP transcription activator that enhances plant tolerance to multiple stresses by positively regulating ABA signaling and fine-tuning cellular levels of ROS. But the molecular mechanism of how ABP9 is regulated in ABA-mediated stress responses remains unknown. Here, we report the identification of ABP9-interacting NCP1 and its roles in drought response, ABA signaling and ABP9 regulation. NCP1 (ABP9 Complex Protein 1) was identified by yeast two-hybrid screening of a maize cDNA library of transcripts prepared from 17dpp immature embryos using ABP9 as the bait. The interaction between NCP1 and ABP9 was confirmed by yeast two hybrid, bimolecular-fluorescence complementation and co-immunoprecipitation assays. Data base homology search revealed that NCP1 shares sequence similarities with NINJA family proteins including AFPs and NINJA/AFPH2 which share three highly conserved domains. But, unlike NINJA and AFPs, NCP1 lacks the conserved domain containing an EAR motif which has been proved to be a hallmark of transcriptional repressors. NCP1 and ABP9 are co-localized in nucleus, and *NCP1* and *ABP9* are co-induced with similar patterns in maize by ABA and osmotic stress, respectively. NCP1 overexpressing Arabidopsis plants, however, exhibited reduced sensitivity to ABA in stomata closure, elevated stress- and ABA-induced ROS accumulation, down-regulated expression of ABA/stress-responsive genes, and decreased drought tolerance. Transient assay in maize protoplasts showed that NCP1 inhibits the activity of ABP9 in activating ABRE-mediated reporter gene expression, and this is not due to the change of ABP9 stability as verified by Western blot, indicating that NCP1 functions as a transcription repressor. This notion is further supported by the observation that NCP1 antagonizes the function of ABP9 on ABA signaling and drought tolerance enhancement, ROS reduction and up-regulation of ABP9 target genes in transgenic plants double overexpressing ABP9 and NCP1 in comparison with that overexpressing ABP9 alone. These data demonstrate that NCP1 is a EAR motif-lacking new member of a subfamily NINJA proteins involved in ABA signaling, and functions as a negative regulator of ABA and stress responses through interacting with and inhibiting the activity of transcription factor ABP9. Possible mechanisms of NCP1 action as a transcription repressor will be discussed.

Funding acknowledgement: National Basic Research Program of China (2006CB100102) and China National Key Progam on Transgenic New Variety Breeding (2011ZX08003-004)

P79

Mapping novel genes of carbon partitioning using the Maize SNP50 chip

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The movement of sugars in plants is crucial to growth, development and reproduction; however, genetic control of carbon partitioning remains poorly understood. We have initiated a search for additional genes that impact this complex process in maize and identified a large number of mutants with apparent defects in sugar movement. We present preliminary mapping of 20 of these genes using bulked segregant analysis and the Illumina Maize SNP50 microarray. Of the 56,000 SNPs on the array, 27,200 SNPs proved informative for analysis of B73 X Mo17 F2 populations and 19,700 proved informative for W22 X B73 F2 populations, allowing mapping to intervals as small as 1 Mb on pooled DNA of up to 87 individuals.

Funding acknowledgement: National Science Foundation (NSF)

P80

Metabolic map of mature maize kernels

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Metabolites in maize kernels are of importance associated with not only nutritional values but also physiological properties such as maturation, desiccation, and germination; however, the information about the comprehensive metabolome in maize kernels is limited. In this study, we identified 210 metabolites in mature kernels of 14 representative maize lines using a non-targeted metabolomic profiling approach. Further statistical analysis revealed that 75 metabolites were significantly variable among those tested lines, and certain metabolites out of the detected 210 metabolites played critical roles in distinguishing one line from another. Additionally, metabolite-metabolite correlation analysis dissected key regulatory elements or pathways involved in metabolism of lipids, amino acids and carbohydrates. Furthermore, an integrated metabolic map constructed with transcriptomic, proteomic and metabolic data uncovered characteristic regulatory mechanisms of maize kernel metabolism. Altogether, this work provides new insights into the maize kernel metabolome that would be useful for metabolic engineering and/or molecular breeding to improve maize kernel quality and yield.

Funding acknowledgement: National Transgenic Plant Special Fund, China (2011ZX08012-002 and 2013ZX08012-002).

P81

Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights

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We present a comprehensive study of maize metabolism, combining genetic, metabolite and expression profiling methodologies to dissect the genetic basis of metabolic diversity in maize kernels. A total of 983 metabolites were quantified in 702 maize genotypes planted at multiple locations. Metabolite-based genome-wide association mapping (GWAS) identified a total of 1459 significant locus-trait associations ($p \leq 1.8 \times 10\text{-}6$) across three environments. Most (58.5%) of the identified loci were supported by expression QTL, and some (14.7%) were validated by linkage mapping. Potential causal variants of five candidate genes were identified by re-sequencing and candidate gene association analysis. Function of two of the five genes was validated by mutant and transgenic analysis, respectively. All data are available as a public resource to aid functional studies and interpretation of GWAS findings. A number of the found metabolites associated with kernel weight can be used as biomarkers to facilitate genetic improvement of maize.

Funding acknowledgement: National High Technology Research and Development Program of China(863)

P82

MicroRNA transcriptomic analysis of heterosis during maize seed germination

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Heterosis has been utilized widely in the breeding of maize and other crops, and plays an important role in increasing yield, improving quality and enhancing stresses resistance, but the molecular mechanism responsible for heterosis is far from clear. To illustrate whether miRNA-dependent gene regulation is responsible for heterosis during maize germination, a deep-sequencing technique was applied to germinating embryos of a maize hybrid, Yuyu22, which is cultivated widely in China and its parental inbred lines, Yu87-1 and Zong3. The target genes of several miRNAs showing significant expression in the hybrid and parental lines were predicted and tested using real-time PCR. A total of 107 conserved maize miRNAs were co-detected in the hybrid and parental lines. Most of these miRNAs were expressed non-additively in the hybrid compared to its parental lines. These results indicated that miRNAs might participate in heterosis during maize germination and exert an influence via the decay of their target genes. Novel miRNAs were predicted follow a rigorous criterion and only the miRNAs detected in all three samples were treated as a novel maize miRNA. In total, 34 miRNAs belonged to 20 miRNA families were predicted in germinating maize seeds. Global repression of miRNAs in the hybrid, which might result in enhanced gene expression, might be one reason why the hybrid showed higher embryo germination vigor compared to its parental lines.

P83

Molecular Characterization of the Restorer-of-Fertility Locus Rf3 of the S-type Cytoplasmic Male Sterility in Maize

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Cytoplasmic Male Sterility (CMS) is a maternally transmitted defect in pollen production and has been widely used in hybrid seed production. The fertility of the CMS-S could be restored by the Restorer Fertility 3 (Rf3) and the fertility restoration was shown to be associated with the suppression of the chimeric ORFs in the mitochondrial. The Rf3 locus was initially mapped on the long arm of chromosome 2. In this study, we reported the fine mapping of the Rf3 locus using a homogeneous population approach and the functional analysis of the candidate genes. The homogeneous population was developed by backcrossing the F1 (Rr) as male to the recurrent male sterile female parent (rr). Because of the gametophytic nature of the CMS-S, only the R gametes were fertile and the r gametes were completely excluded from fertilization. The BC1F1 consisted of only Rr genotype and all BC1F1 individuals should then be male fertile. We grew out about 10,000 BC1F1 individuals in Hainan province in 2012 and 5000 in Beijing in 2013 and none of the plants was male sterile, indicating the feasibility of this mapping method. We then screened with molecular makers a BC1F1 population of about 7000 individual plants and mapped the Rf3 locus to a 97kb region in the B73 genome. Several candidate genes located in this region and one of them encoded a 325-aa pentatricopeptide repeat protein. Interestingly, nucleus-encoded PPR repeat genes have been shown to modify the expression of CMS-associated regions in different plant species, including rice, petunia and Brassica. Furthermore, a mitochondrial targeting sequence and a cleavage site in this 325-aa PPR protein were identified, suggesting its possible involvement in the mitochondrial RNA processing. All evidence indicated that Rf3 might be a PPR protein and this 325-aa PPR protein was an ideal candidate for Rf3.

P84

Morphological and Transcriptomic Analysis of a Male Sterile Line in Maize (*Zea Mays L.*)

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Maize is one of the most important crops all over the world. The maize hybrid seed is widely used because of the heterosis. Detasseling is a key step during hybrid seed production. Manual detasseling is time and money consuming, and also affected by weather. In addition, detasseling by machine could decrease the yield for the over cut. Stringent male sterile line is the best choice for maternal parent. Here a maize male sterile line was obtained by distant hybridization. The anther in male flower was replaced by small lamma with a few fine hairs. Occasionally, a silk initiated at the base of lamma and elongated out of the flower. Meanwhile, each seed had more than one silk and made up the hairy ear. The male sterile could be observed during anther identity formation. The anther primordium first proliferated ball-shaped mold and then expanded as a thin piece object rather than four prisms as the normal one did. Genetic analysis revealed a single recessive gene controlled this phenotype. A BC1F1 population was used for the genetic mapping. The gene was located at the long arm of chromosome 6 based on the B73 RefGen_v2 sequence. RNA-seq of the mutated male/female flower was performed. Comparing to wild type, around one hundred of genes were abnormally expressed. Several of them were involved in the ABC flowering model, indicating a flowering regulation role of the mutated gene.

Funding acknowledgement: National Science Foundation (NSF)

P85

Natural Variation Associated with Seed Composition Traits to Improve Micronutrient Efficiency

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It is known that the world needs to grow and produce more food than past thousands years and boost nutrient efficiency in order to feed over 7 billion continously increasing population. Therefore, it is our challenge to grow more nutritious food with less agronomical inputs in the next decades. Micronutrient deficiency affects 35% of the world population and causes stunted growth, weaker immunity, and impaired cognition, especially in developing countries. There is a wide range of natural variation in seed composition of many studied food plants. Therefore, identifying, developing, and using high micronutrient seed content traits are of interest for plant biology and human nutrition. In this study, we investigated genes and genetic variability that control seed Zn, seed weight, and seed proteins in recombinant inbred lines and genotypes differ in these traits. Our second objective was to develop a non-destructive, low-cost technique for detecting individual single-seed composition. Our results showed that NIR-Spectroscopy shows potential for sorting multiple seed traits. Together, this set of experiments should allow us to begin to understand seed micronutrient composition variation in major staple food crops such as maize and beans. The current status of this project and further detailed results will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P86

Overexpression of the Maize *GRF10*, an Endogenous Truncated GRF Protein, Leads to Reduction in Leaf Size and Plant Height

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It has long been thought that growth-regulating factors (GRF) gene family members act as transcription factors to play important roles in multiple plant developmental processes. However, the recent characterization of Arabidopsis *GRF7* showed that it functions as a transcriptional repressor of osmotic stress-responsive genes. This highlights the complex and diverse mechanisms by which different GRF members employ to take action. In this study, the maize *GRF10* was functionally characterized to improve this concept. The deduced ZmGRF10 protein retains the N-terminal QLQ and WRC domains, the characteristic regions as protein-interacting and DNA binding domains, respectively. However it lacks nearly the entire C-terminal domain, the regions executing transactivation activity. Consistently, ZmGRF10 protein maintains the ability to interact with GIFs proteins, but lacks transactivation activity. Overexpression of *ZmGRF10* in maize led to a reduction in leaf size and plant height through decreasing cell proliferation, whereas the yield-related traits were not affected. Transcriptome analysis revealed that the multiple biological pathways were involved in the altered phenotypes in *ZmGRF10* overexpression plants, including a few of transcriptional regulatory genes which have been demonstrated to have important roles in plant growth and development. We propose that ZmGRF10 aids fine-tuning the homeostasis of the GRF-GIF complex in the regulation of cell proliferation.

Funding acknowledgement: National Transgene Research and Industrialization Project of China (2011ZX08003-003-00A),National Program on Key Basic Research Project of China (973Program:2014CB147300)

P87

Overexpression of ZmSnRK2.11, an SNF1 type serine threonine protein kinase in maize, decrease tolerance to osmotic stress in Arabidopsis

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Drought and salinity are major factors limiting crop productivity and quality. Sucrose non-fermenting 1-related protein kinases 2 (SnRK2) represents a unique family of protein kinase in abiotic stress signaling transduction in plants. In our study, a novel SnRK2 gene, ZmSnRK2.11, which harbors eight introns in its coding region, was cloned from maize. We demonstrate that ZmSnRK2.11 expresses differentially in various organs of maize plants and it is up-regulated by high-salinity, dehydration and ABA treatment. The ZmSnRK2.11 cDNA was fused to a Yellow fluorescent protein (YFP), and the subcellular localization experiment shows that the fusion protein was localized in the cell membrane, cytoplasm and nucleus. Overexpressing ZmSnRK2.11 in transgenic Arabidopsis had enhanced sensitivity to drought and salt stresses, and the results were supported by physiological data, including increased rate of water loss, reduced relative water content, delayed stoma close and increased MDA content. Further analysis indicates that transgenic plants show decreased transcription of the stress-related genes under normal or high-salinity conditions. Taken together, our results imply that ZmSnRK2.11 potentially play a role as a negative regulator in response to osmotic stress.

P88

Phenotype to genotype with “forward-genetic Mu-seq”

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Mu-Seq technology, initially developed for the construction of the UniformMu public resource, can also be adapted for fast, efficient genetic analysis of phenotype-genotype correlations. Here we present and use “forward-genetic Mu-Seq” for high-throughput dissection of Mu-insertion profiles of multiple families with like phenotypes. For kernel phenotypes, 12 lines were selected from the UniformMu population, segregating families were developed, and a Mu-seq grid was constructed that integrated phenotypes with Mu-seq profiles. We simultaneously tracked 282 segregating Mu insertions in 141 individual plants using this approach and tested for co-segregation with kernel phenotypes. Seven lines showed genes that cosegregated with the selected phenotypes including at least five that share putative roles in organellar mRNA processing. A high percentage of visible-mutant phenotypes cosegregated with insertions in coding sequence rather than with Mu elements in introns or 5’utr sequences. These results and other data have led us to focus on Mu insertions in translated regions of genes. We are also adapting forward-genetic Mu-seq to test the genetic basis for non-lethal seed phenotypes. Kernel phenotypes are assessed on selfed ears and seedling phenotypes are assessed by planting the kernals from selfed ears following the first generation and screening for vegetative mutants. Forward-genetic Mu-seq allows high-throughput dissection of kernel and vegetative phenotype-genotype relationships.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P89
Poster has been removed from the program.

P90
Single reaction multiplex TaqMan assay for simultaneous multiple SNP and CNV data acquisition

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Probe-based PCR is widely used for SNP (single nucleotide polymorphism) genotyping and gene copy variation in molecular crop breeding due to its simplicity, sensitivity and cost-effectiveness. However, the multiplex capability of TaqMan probe-based PCR is normally limited to one allele or one copy number data per two fluorescence channel (one channel for each diploid allele, or one channel for reference and one for target). Current generation of qPCR instruments typically have 5–6 optical channels. We present here fluorescent probe sets and reagent to take advantage of the multichannel capability of the instruments that allows detection of multiple targets in a single reaction that utilize the multiple channels for multiple data point simultaneously. Multiplex in a single of a closed-tube PCR offers a new level of efficiency in data through put and reduction in cost per data point that is beneficial in a molecular breeding production environment. This selective primer and probe sequence design and use different probes labeled with the different fluorophore. We further optimize the PCR reaction that allows multiplex amplification that each target does not interfere with others. The optimized multiplex reaction is able to obtain the same result as separate reactions. In conclusion, we have validated the multiplex capacity, the simplicity and accuracy of multiplex genotyping and copy number variation (CNV) in single reactions.

P91

Study about the specific fragment accompanied with fertility restored F1 of CMS-C male sterile line in maize(zea mays) by SSR

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The character of cytoplasmic male sterile (CMS) in plant is more favourable for the seed production of commercial Hybrids. There are three types of CMS in maize, they are CMS-C ,CMS-T and CMS-S. It is well known that CMS-C could be the most potential type in practice. But the research progresses about CMS-C fall behind of the CMS-T and CMS-S, and the mechanism of abortive and fertility restoration remain unclear, so it is considered that the mechanism of CMS-C may be more complicated. This study display some curious phenomena, the first is that some test lines have an opposite fertility restoration to one set of isoplasm-allonuclear CMS-C male sterile line .The second is that the fertility restored F1 produced a specific band which did not be detected in their parents and the sterility maintained F1 by SSR technology .

P92

Sucrose Transporter1 function is essential for long-distance transport of sucrose in maize

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Plants assimilate carbon dioxide within leaves and convert it into sucrose, the principal form of carbohydrate transported long distance in maize. Sucrose is loaded into phloem tissues in leaves by sucrose transporters (SUTs), distributed throughout the plant through the veins, and unloaded into sink tissues, e.g., roots, stems, and ears. SUTs are a family of integral membrane proteins that transport sucrose across a membrane using energy stored in the transmembrane proton gradient. We have identified seven *Sut* loci in maize and are characterizing their functions through both molecular and reverse genetic approaches. The *Sut1* gene is expressed in mature source leaves and sink tissues, and loss-of-function mutant analyses demonstrated that the gene functions in phloem loading. To further understand the functions of the *Sut1* gene, two different fluorescent reporter protein constructs were transgenically expressed in maize. One is a promoter reporter construct driving expression of a red fluorescent protein (pSut1-RFP), and the second is a translational fusion of the yellow fluorescent protein (YFP) to the C-terminus of the SUT1 protein, under the native regulatory sequences (gSUT1-YFP). The gSUT1-YFP construct was crossed into the *sut1* mutant background and determined to functionally complement the mutation, indicating the YFP fusion did not inhibit SUT1 function, and that the transgene is expressed in the correct location to provide the missing function. Fluorescent microscope investigations of the cellular and subcellular expression patterns of the genomic and promoter fusion constructs will be presented. Additionally, immuno-transmission electron microscope studies were used to confirm the subcellular protein localization data. Collectively, these studies provide a detailed understanding of where the *Sut1* gene is expressed, and expand our knowledge of the multiple roles of SUT1 in controlling transport of sucrose throughout the plant. This work provides the first evidence for a SUT functioning in sucrose phloem loading in monocots.

Funding acknowledgement: National Science Foundation (NSF)

P93

The *defective kernel5* (*dek5*) locus encodes a plant specific protein required for grain-fill and chloroplast function

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Mutant alleles of the *defective kernel 5* (*dek5*) locus condition a severely reduced starchy endosperm with a shrunken phenotype similar to starch biosynthetic mutants such as *brittle1* and *brittle2*. Unlike endosperm starch biosynthetic mutants, *dek5* mutants can produce a normal embryo that develops pale green seedling leaves with occasional white stripes. The pale green seedling phenotype of *dek5* is seedling lethal and suggests the gene is required for chloroplast function. Transmission electron microscopy (TEM) of *dek5* leaf tissue revealed that the mutants have significantly enlarged chloroplast when compared to normal siblings. Genetic mapping of *dek5* with approximately 700 mutant kernels from a Mo17 × *dek5-25* F2 population narrowed the locus to a 460 kbp interval on chromosome 3. By co-localizing the genetic map position with transposon flanking sequence tags (FSTs) from the *dek5-25* mutant, we identified a candidate insertion at the first exon of a gene with unknown function. Complementation tests with additional alleles from UniformMu reverse genetics resources confirmed the identity of the *dek5* gene. *dek5* encodes a 2,123 amino acid protein from a 30 kbp gene. Bioinformatics analysis of the DEK5 protein sequence predicts the protein is targeted to the chloroplast. Orthologous *dek5* genes are found in the genomes of all completely sequenced photosynthetic organisms, but*dek5* genes are not found in other species. Based on these data, we hypothesize that *dek5* compromises plastid function in the amyloplast of the starchy endosperm as well as the chloroplast in seedling leaves.

Funding acknowledgement: United States Department of Agriculture (USDA), China Scholarship Council (CSC)

P94

The genetic architecture of rind penetrometer resistance in two maize recombinant inbred line populations

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Maize is the most important cereal crop around the world, and provides staple food for billions of people. Stalk lodging is a main factor to influence the stability of maize production. Rind penetrometer resistance (RPR) is an effective method to evaluate maize stalk strength, which is highly related with stalk lodging resistance. In this study, two recombination inbred line (RIL) populations were constructed from crosses between B73 and By804, H127R and Chang7-2, and genotyped using 3072 single nucleotide polymorphisms (SNPs) and phenotyped in multiple locations and years. In total, 2 and 4 QTL were identified for RPR, and explained 27.82% and 38.05% of phenotypic variations in By804/B73 and Chang7-2/ H127R RIL populations, respectively. None common QTL detected between two RIL populations demonstrates the complex nature of RPR. The largest QTL for RPR, *qRPR3-1*, with explained phenotypic variation of 18.9%, were located between SNP makers PZE-103123325 and SYN23245 on chromosome 3, which covering 3.6-Mb genomic region. Further haplotype analysis narrowed this locus down to 3.1 Mb using 10 SNPs in the target regions with 141 RILs. According to the subsequent function annotations of genes within the QTL interval, we inferred four potential candidate genes associated with cell wall component, which were indirectly correlated with stalk strength. These results provide valuable knowledge of cloning the genes controlling RPR, and the manipulation of the improvement of maize stalk strength via molecular breeding.

Funding acknowledgement: National Natural Science Foundation of China

P95

The genetic architecture of zinc and iron content in maize grains as revealed by QTL mapping and meta-analysis

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Micronutrient malnutrition, especially zinc (Zn) and iron (Fe) deficiency in diets, has aroused worldwide attention. Biofortification of food crops has been considered as a promising approach for alleviating this deficiency. Quantitative trait locus (QTL) analysis was performed to dissect the genetic mechanism of Zn and Fe content in maize grains using a total of 218 F2:3 families derived from a cross between inbred lines 178 and P53. Meta-analysis was used to integrate genetic maps and detect Meta-QTL (MQTL) across several independent QTL researches for traits related to Zn or Fe content. Five significant QTLs and 10 MQTLs were detected. Two informative genomic regions, bins 2.07 and 2.08, showed a great importance for Zn and Fe content QTLs. The correlation between Zn and Fe level in maize grains was proposed by MQTLs as 8 of the 10 involved both traits. The results of this study suggest that QTL mapping and meta-analysis is an effective approach to understand the genetic basis of Zn and Fe accumulation in maize grains.

Funding acknowledgement: National High Technology Research and Development Program ("863" Program) of China (2011AA10A103-3).

P96

The maize glossy26 gene encodes an enoyl-CoA reductase involved in the biosynthesis of epicuticular waxes

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Epicuticular waxes coat the surfaces of aerial portions of plants where they play roles in resistance to biotic and abiotic stresses. Mutations affecting the biosynthesis or deposition epicuticular waxes present a "glossy" phenotype. A large collection of *gl* mutants has been generated, which in combination define about 20 distinct loci. In plants, epicuticular waxes are derivatives of very-long-chain fatty acids (VLCFAs; ≥20 carbons), which are synthesized by a membrane-associated fatty acid elongation system that catalyzes four cyclic enzymatic reactions: condensation, reduction, dehydration, and a second reduction. The single copy maize gene, GRMZM2G481843 is a homolog of the *yeast* gene TSC13 and the *Arabidopsis* gene CER10, both of which encode enoyl-CoA reductase (ECR), which catalyzes the second reduction during VLCFA elongation. A bioinformatic candidate gene approach was used to demonstrate that the previously defined *glossy26 (gl26)* gene of maize is GRMZM2G481843 and therefore putatively encodes an ECR. By analyzing RNA-seq data, ~3,000 differentially expressed genes (DEGs) were identified between the gl26 mutant and wild-type controls. These DEGs are significantly enriched in pathways involved in primary and secondary metabolism, including wax metabolism and wax transport. Combining the DEG list, expression patterns across organs and tissues with sequence similarity we identified a set of putative wax-related genes. This gene set is being used to clone additional glossy loci via candidate gene approaches.

Key words: *gl26*, candidate gene approach, ECR, RNA-seq analysis

Funding acknowledgement: National Science Foundation (NSF)

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The role of the basal endosperm transfer cells in the protection of the kernel against pathogens

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The transfer cell layer of the maize endosperm has a crucial role in the grain filling process. Transfer cells (TC) are positioned at the base of the endosperm, facing the vascular strands that release nutrients in the pedicel. TC develop modified cell walls containing a dense network of ingrowths that greatly increase the exchange surface, thus facilitating the uptake of nutrients from the maternal apoplast. This need to facilitate the transit of solutes has, however, a potential drawback, pathogens infecting the mother plant could easily ingress into the developing seeds. Not surprisingly some TC specific genes has been putatively correlated with a role in plant defence, and in a limited number of cases this role has been supported by at least in vitro activity assays. The real importance of the TC layer in the health of the maize progeny it is however unknown, even the relative contribution of the TC gate to the entrance of pathogens into the seed remains to be elucidated. In this work we will show our progresses in the following research lines:
(1) Monitoring the infection of immature maize kernels by the maize pathogens Aspergillus niger, Aspergillus flavus and Fussarium moniliformis, for this we have designed PCR diagnostic tests that can detect quantitatively fungal DNA in maize DNA down to the level of femtograms.
(2) Identifying genes induced at the transfer cells by the pathogens.
(3) Characterizing antipathogen genes induced by the TC-specific transcriptional activator ZmMRP-1 in heterologous systems.

Funding acknowledgement: Bill and Melinda Gates Foundation to G.H.

P98

The *ZmSAD1* gene is associated with natural variations of stearic acid composition in maize kernels

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Maize oil is highly regarded as healthy vegetable oil due to its low saturated fatty acid compositions. The manipulation of the ratio between unsaturated and saturated fatty acid compositions plays a critical role on the improvement of oil quality in maize kernels. Candidate-gene association analysis and linkage analysis demonstrate that the gene encoding stearoyl-ACP-desaturase (*ZmSAD1*) within a quantitative trait locus (QTL) was associated with stearic acid composition and conversion in maize kernels. *ZmSAD1* alleles associated with reduced transcript expression correlate with higher stearic acid composition and lower ratio between unsaturated and saturated fatty acid compositions. These results were further validated by silencing and over-expression *ZmSAD1* in Arabidopsis, and in turn indicated that *ZmSAD1* regulated the natural variation of the ratio between unsaturated and saturated fatty acid compositions via transcription regulation. In addition, no positive selection for *ZmSAD1* occurred during maize domestication and high-oil maize artificial selection, consistent with that fatty acid compositions were not the target of selection during both progress.

P99

Transcriptome analysis of maize in response to *Fusarium graminearum* infection

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Gibberella stalk rot, caused by *Fusarium graminearum*, is one of the most devastating soil-borne diseases in maize. Two QTLs, the major *qRfg1* (A) and the minor *qRfg2* (B), were detected to confer resistance to *Gibberella* stalk rot. For each QTL, we developed advanced backcross generations and selected those individuals with the shortest donor region and the highest recovery rate of the recurrent parent. Two such individuals, one with *qRfg1* and the other with *qRfg2*, were then crossed and selfed to produce four near-isogenic lines (NILs), named as NIL-AABB, NIL-AAbb, NIL-aaBB, and NIL-aabb. RNA-seq analysis was conducted for above four NILs at 0, 6, and 18 hours after *F. graminearum*inoculation. Differentially expressed genes in and between different NILs were identified, in which common up-regulated genes among four NILs were amounted to as many as 1,228. Enriched go terms for these common up-regulated genes in biological processes were found to be regulation of plant-type hypersensitive response, systemic acquired resistance, jasmonic acid mediated signaling pathway, response to chitin, and MAPK cascades, etc. The result implies that the basal resistance mechanisms or pathways are common among the four NILs, in spite of the presence or absence of resistance QTLs. Comparative transcriptome analysis showed that a total of 1,070 genes were exclusively up-regulated in NIL-AAbb compared to NIL-aabb after *F. graminearum* infection. These *qRfg1*-dependent up-regulated genes are predominantly represented by resistance-related transcription factors, kinases, and many types of transferases. A total of 824 genes were exclusively up-regulated in NIL-aaBB compared to NIL-aabb. Pathway analysis showed that protein processing in endoplasmic reticulum, flavonoid biosynthesis, and plant hormone signal transduction were significantly enriched in these *qRfg2*-dependent up-regulated genes. Whereas, in NIL-aabb, flavonoid biosynthesis and plant hormone signal transduction pathways were significantly represented in the down-regulated genes.

Funding acknowledgement: the National Basic Research '973' program of China

P100

Ultra-high-density markers reveal the genetic basis of grain yield heterosis in an "immortalized F_2" maize population

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Heterosis is a fundamental biological phenomenon which continues to play a critical role in boosting global grain yields. Notwithstanding our limited insight into the genetic and molecular basis of heterosis, it has been exploited extensively using different approaches. In this study, we investigated the genetic underpinnings of grain yield and yield components using "immortalized F_2" and recombinant inbred line populations (RILs) derived from an elite hybrid Yuyu22. A high density linkage map consisting of 3184 bins was used to assess (1) the additive and additive-by-additive effects determined using recombinant inbred lines; (2) the dominance and dominance-by-dominance effects from mid-parent heterosis dataset; and (3) all types of genetic effects in an "immortalized F_2"population. Compared with a low density simple sequence repeat (SSR) map, the high density bin map identified more quantitative trait loci (QTLs), with higher LOD scores and higher precision of QTL detection. The high density bin map showed that, among all traits, dominance was more important to heterosis than other genetic effects. The importance of overdominance or pseudo-overdominance was proportional to the amount of heterosis. In addition, epistasis contributed to heterosis as well. Phenotypic variances explained by both single loci and di-genic epistasis were approximately equal to the broad-sense heritabilities of the observed traits. Comparison of analyzed results in "immortalized F_2" population to those in mid-parent heterosis dataset indicated identical genetic basis for heterosis and trait performance, and heterotic loci were likely part of overdominance QTLs of trait performance.

Funding: National Hi-Tech Research and Development Program of China, the National Basic Research Program of China

P101

Unraveling the regulatory network of maize defense response to *Ostrinia furnacalis*

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Asia Corn borer (*Ostrinia furnacalis*) has been a major cause of yield loss of maize production in China. A better understanding of the maize-*Ostrinia furnacalis* interaction at the molecular level would be necessary for engineering the maize defense response to herbivory genetically. In this study, by using the illumina HiSeq2000 platform, we systematically surveyed the transcriptomic changes of B73 seedling leaves in response to *Ostrinia furnacalis*, and also to jasmonic acid, which plays a major role in plant defense response to herbivores. About 1500 genes regulated by *Ostrinia furnacalis* infestation (RPKM fold change>2, $p<0.05$) mainly fall into functional categories including jasmonate signaling components, defense response (e.g. proteinase inhibitors, ribosome inactivating proteins), cell wall remodeling, secondary metabolism, etc. We also observed a strong correlation of gene expression alteration patterns between *Ostrinia furnacalis* and JA treatment, suggesting that jasmonates control the expression of a large proportion of insect-induced genes, just as in other plant species such as *Arabidopsis*.

Funding acknowledgement: National Science Foundation of China (NSFC)

P102

Use Of Forward Genetic Screens In Rice For Trait Discovery

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A significant challenge we face is increasing agricultural productivity to feed a growing population. Science and technology have the potential to impact global food production through the discovery of traits that deliver higher yields per acre, allow cultivation of crops under stress conditions, and protect crops against pest infestations and diseases. In addition to being a multi-billion dollar crop grown in more than a hundred countries, rice is a very useful functional genetic tool for trait discovery: rice has a relatively small genome size, high throughput transformation methods, a complete genome sequence, and it is co-linear with other plant genomes such as corn. We have developed a population of several thousands of activation-tagged rice lines for use in genetic screens to identify genes that improve water and nitrogen use efficiency. We have screened and identified a number of lines with improved drought tolerance and nitrogen use efficiency. Candidate genes are being identified and further validated by transgenic expression in rice to confirm the improved traits.

P103

Wrinkled kernel1 encodes beta-tubulin5 protein that is required for sister chromatin segregation and endosperm development in maize

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The large endosperm of maize not only is a nutritive tissue for embryo development, but also accounts for the primary food products and industrial applications, which make maize the central crop of agriculture. It is clear that maize endosperm develops from the fertilized central cell, originates with repeated free-nuclear divisions followed by cellularization, and subsequently forms four differential cell types, yet the regulation of maize endosperm development remains poorly understood. Here, we report the characterization of a wrinkled kernel1 (wrk1) mutant. The endosperm in the Wrk1 mutant was deeply furrowed and affected by gene dosage, whereas the seedling was small but vigorous. The section of kernel in 10 DAP, 12 DAP and 16 DAP showed that the effects of Wrk1 mutation are manifest in formation of starch endosperm cells and transfer layer cells. Immunostaining of spindle by beta-tubulin antibody in anaphase of mitosis suggested that Wrk1 mutant was defective in sister chromatin segregation resulting in chromosome bridge and chromosome lagging. Using positional cloning strategy, we mapped the causative mutation in a 350-kb region, which contains three predicted gene models. A gene model encoded beta-tubulin5 protein showed a C to T transition leading to a premature stop codon in 3' end. The truncated ZMTUBB5 completely lacked C-terminal tail of isotype-defining region. These results indicated that Wrk1 encodes the ZMTUBB5 which is essential for endosperm development in maize.

P104

X1-homologous genes family as central components in biotic and abiotic stresses response in maize (Zea Mays.L.)

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X1-homologous genes (XHS) encode plant specific proteins containing three basic domains (XH, XS, zf-XS). In spite of their physiological importance, systematic analyses of ZmXHS genes have not yet been explored. In this study, we indicated the isolation and characterization of ZmXHS genes in whole-genome wide. A total of 10 members of this family were identified in maize genome. The 10 ZmXHS genes were distributed on seven maize chromosomes. Multiple alignment and motif display results revealed that most ZmXHS proteins share all the three conserved domains. Putative cis-elements involved in abiotic stress responsive, phytohormone, pollen-specific and quantitative, seed development and germination, light and circadian rhythms regulation, Ca2+-responsive, root hair cell-specific and CO2-responsive transcriptional activation were observed in the promoters of ZmXHS genes. Yeast hybrid assay revealed that the XH domain of ZmXHS5 was necessary for interacts with itself and ZmXHS2. Microarray data showed that the ZmXHS genes had tissue-specific expression pattern in the maize developmental steps and biotic stresses response. QRT-PCR analysis results indicated that, except ZmXHS9, the other nine ZmXHS genes were induced in the seedling leaves by at least one of the four abiotic stresses applied.

Funding acknowledgement: Beijing Municipal Science and Technology Commission, Beijing Nova Program, Youth Foundation of Beijing Academy of Agriculture and Forestry Sciences

P105

Activation of cytokinin signaling in maize leaves uncovers a potential organogenic expression program at the grass leaf margin

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The maize leaf consists of distinct compartments polarized along the proximal-distal (P-D) growth axis. The proximal sheath is separated from the distal blade by the auricle and ligule. To better understand the molecular mechanisms which specify this P-D pattern of growth, we are studying the semi-dominant, gain-of-function *Hairy Sheath Frayed1* (*Hsf1*) mutation which conditions ectopic outgrowths of proximal tissue – sheath, auricle and ligule – from the distal blade margin. The *Hsf1* mutant phenotype is caused by specific missense mutations in the maize cytokinin (CK) receptor *Zea mays Histidine Kinase1* (*ZmHK1*) which lead to inappropriate CK signaling in developing leaf primordia. To investigate how CK signaling specifies proximal tissue outgrowths, we performed transcriptome profiling on blade margins collected using laser microdissection (LM). LM was used to collect cells from wild-type margins and *Hsf1* margins in regions with and without proximal tissue outgrowths (prongs). Data analysis revealed significant expression changes in about 900 genes in *Hsf1* margins with prongs compared to *Hsf1* margins without prongs or wild-type margins. Surprisingly, a subset of genes differentially expressed in *Hsf1* prongs is analogous to the set of genes known to function downstream of CK signaling that controls compound leaf development in eudicot species like tomato. In tomato, the expression of class I *knox* genes, other transcription factors and CK genes is required to maintain a zone of organogenic activity at the blade margin where leaflet initiation is established. In *Hsf1* developing prongs, one of the genes with the highest fold-change is *liguleless3* (*lg3*), a member of the class I *knox* gene family. Ectopic expression of *lg3* in a *Hsf1* background enhanced the *Hsf1* phenotype, consistent with *lg3* expression promoting prong initiation. We will present our current results exploring the functional similarities between prong growth in *Hsf1* maize leaves compared to compound leaf development in eudicots.

Funding acknowledgement: National Science Foundation (NSF)

P106

An automated Whole Plant Phenotyping System for early vigor trait screening and selection

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As part of Syngenta's abiotic stress trait development pipeline, plants are screened for early vegetative growth and vigor, relying on changes in plant growth, mass (aka biomass), and efficiency of water use as indicators of plant responses to environment. The traditional method for monitoring plant biomass over the growing cycle involves the labor-intensive process of manually weighing each pot on a daily (or more frequent) basis. This is impractical for screening hundreds of plants. Thus we have developed and implemented a Whole Plant Phenotyping System (WPPS) to fully automate the process using a combination of base scales to continuously measure plant weight and a feedback system to enable precise, automated control of soil water content. The WPPS weighs plants automatically as they grow and, taking new measurements for each plant every five minutes, for a total of 288 measurements per plant per day, and adjusts soil water content as needed. This is in contrast to commercially-available systems which bring the plant to the instruments which limits the number of measurements possible per plant per day. Measurements are automatically logged in software which collectively tracks the weight of every plant, allowing subtle changes in plant growth rates to be rapidly detected long before possible with conventional methods. The level of accuracy and granularity supplied by the WPPS permits conclusions not possible with manual weighing or commercially available conveyor systems, and does so without risking damage to the plants or injury to employees or adding the possibility of human error to the calculations. Here we will present some of the technology behind this system and examples of how it is being implemented as a part of Syngenta's agronomic trait R&D pipeline.

P107

Analysis of maize embryo morphogenesis in nine emb mutants.

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The genetic control of maize embryogenesis may be examined by analyzing the effects of embryo-specific mutations on embryo morphogenesis. Mutations were induced by treating maize pollen from the W22 inbred line with ethyl methanesulphonate. The treated pollen was crossed onto W22 females. The resulting kernels were planted and the progeny plants were self-pollinated. The selfed ears were used as the source of kernels to be screened. Ears segregating for embryo specific mutants (emb) were identified by examining under magnification normal-appearing kernels and finding that approximately one-fourth of the kernels contained embryos reduced in size. These are single gene recessive mutants and are likely to be homozygous lethal. The extent of morphogenesis of the mutant embryos was documented by dissection of mature kernels and photography of the mutant embryos. Each mutant was evaluated by comparing the extent of embryo development with the maize embryo developmental stages of Abbe and Stein (1954). The mutants ranged in the extent of their embryo development, from the transition stage to more advanced stages. The nine mutants presented here represent as many as nine loci that appear to specifically affect embryogenesis.

Funding acknowledgement: National Science Foundation (NSF)

P108

Barren inflorescence3, a novel semi-dominant maize mutant defective in meristem initiation and maintenance

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The coordinated regulation of genes and hormones is required for the initiation and maintenance of groups of pluripotent stem-cells called meristems, whose activities regulate the formation of maize inflorescences, the uppermost male tassel and the lateral female ears. We are characterizing a novel semi-dominant maize mutant, called *Barren inflorescence3 (Bif3)*, whose severe inflorescence phenotype suggests it encodes a key gene in meristem development. Defects in *bif3* gene function result in the formation of tassels with shortened central spikes and ears that are stunted and partially bald. Detailed morphological analysis of *Bif3* mutant inflorescences shows a significant decrease in the number of paired spikelets, which are instead often replaced by single spikelets or by barren regions completely devoid of spikelets. Furthermore, the inflorescence meristem often appears to collapse during development, thus resulting in smaller tassels and ears. These phenotypes suggest that *bif3* is required for both the initiation and, in particular, the maintenance of meristem activity. Using a map-based cloning approach we narrowed the region containing the *bif3* locus to 0.09 Mb on chromosome 2. Among the candidate genes found in this small window, there is a gene related to *WUSCHEL*, which is known to be involved in meristem maintenance in Arabidopsis. This gene is significantly unregulated in *Bif3* mutant inflorescences, suggesting that its misexpression may underlie the observed phenotype. Genetic and molecular interactions with other genes involved in meristem maintenance are under investigation.

Funding acknowledgement: National Science Foundation (NSF)

P109

BETL9 and BETL9like, two genes encoding non-specific lipid transfer proteins with a complementary transcription pattern at the outer surface of the developing maize endosperm

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BETL9 was identified in the course of a screening for maize endosperm transfer cell-specific genes. BETL9 encodes a non-specific lipid transfer protein (nsLTP) very similar to the product of the barley transfer cell specific end-1 gene. In situ hybridisation analysis confirmed that the BETL9 gene is indeed specifically transcribed in the basal endosperm transfer cell layer (BETL) starting 5 days after pollination. However, immunolocalisation data revealed that the BETL9 protein accumulates in the maternal placento-chalaza cells located opposite to the BETL, suggesting that is the place where it exerts its still unknown function. We have identified a second maize gene very similar in sequence to BETL9 and called it BETL9like. In situ hybridisation showed that BETL9like is exclusively expressed in the aleurone cell layer and the border between the expression domains of the BETL9 and BETL9like genes also marks the boundary between the two tissues covering the surface of the developing endosperm: the BETL and the aleurone. The BETL9 and BETL9like promoter sequences fused to the GUS reporter gen, accurately reflected the expression pattern observed for the endogenous genes in transgenic maize. Finally, we have identified in the Arabidopsis genome a set of four genes orthologous to BETL9 and BETL9like and analyzed the activity of their promoters fused to the GUS reporter gen in Arabidopsis transgenic plants. As in the case of the maize genes, the Arabidopsis genes showed highly complementary expression patterns.

Funding acknowledgement: Spanish Ministerio de Ciencia e Innovacion to G.H. (BIO2009-11856 and BIO2012-39822), Biogemma SAS

P110

Brassinosteroid signaling in maize shoot development

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Brassinosteroid (BR) hormones are critical for many aspects of plant growth, development and stress responses. Arabidopsis and rice show conservation in the key components of BR signaling, however, BR signaling in maize is not well characterized. To develop markers, an RNA-seq analysis was performed on BR deficient *nana1* mutant and several genes were identified as downregulated or upregulated in response to increasing levels of exogenous Brassinolide (BL). We also developed a BES1-YFP transgenic line, and show that BES1-YFP accumulation in the nucleus is BR responsive.
To alter BR signaling we targeted *bri1* and *bin2* with RNAi. BRI1 is a receptor kinase with an extracellular "Island Domain" that binds BR. 5 BRI1 homologs were identified and a *bri1*-RNAi construct was designed using the extracellular domain of the top hit. The *bri1*-RNAi plants showed dwarf stature with altered leaf and auricle morphology. Gene expression analyses suggests that bri1-RNAi disrupted BR signaling. BIN2 is a GSK3-like protein kinase that negatively regulates BR signaling. As such, RNAi suppression is expected to upregulate BR signaling. 10 BIN2 homologs were identified in maize and since there is probable redundancy, as in Arabidopsis, we used the whole cDNA from the top hit for designing an RNAi construct. *bin2*-RNAi suppression caused a range of phenotypic changes from mild to strong. As opposed to *bri1*-RNAi plants, *bin2*-RNAi plants tend to have larger auricles compared to their WT siblings and larger leaves with crenulated leaf margins. Some organs were elongated but unexpectedly stem internodes were shortened. In addition, tassel spikelet density is decreased and spikelet formation is aborted on tassel branch tips. Expression of BES1-YFP in the auricle region and the altered auricle morphology in both *bri1*-RNAi and *bin2*-RNAi transgenic plants suggest that BR signaling is involved in auricle development in maize.

Funding acknowledgement: Iowa State University Plant Sciences Institute, Republic of Turkey Ministry of Education

P111
Cell Wall Histochemistry of Maize Anthers

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Relatively little is known about the cell wall composition of the anther, the male reproductive organ in flowering plants. Because plants lack a germ line until after flowering, the anther is a site of highly coordinated cell division and differentiation as the central most anther lobe cells switch from mitotic to pre-meiotic germ cells. Accompanying germinal development, a series of somatic niche cells differentiate and develop to nurture the growing meiotically-competent cells into mature pollen. Despite extensive anther studies, relatively little is known about anther cell wall composition over the course of development. Through a histochemical approach we characterized a variety of cell wall components using specific cell wall stains across a timeline of anther development in *Zea mays*. Lignin, pectin, and cellulose were all found to be enriched in specific somatic niche cell layers and to vary in concentration across anther development.

Funding acknowledgement: National Science Foundation (NSF), Stanford University

P112
Characterization of miRNAs and their targets in maize seed development

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MicroRNAs (miRNAs) are approximately 20-22 nt non-coding RNAs that play key roles in many biological processes in both animals and plants. To better understand the roles of miRNAs in developing maize seeds, we sequenced small RNAs from 10 days-after-pollination (DAP) seed, 25 DAP embryo and 25 DAP endosperm.
There were 25 conserved miRNAs that were differentially expressed among these tissues. Based on the existence of miRNA*, novel miRNAs were identified and some of them were experimentally validated. In addition to deep-sequencing, miRNA microarray study confirmed the differential expression of several miRNAs in seeds.
To identify cleaved targets of miRNAs, we sequenced the degradome of six tissues, and 70 cleaved targets of 37 miRNAs from 18 miRNA families were identified. Six of these targets were validated by 5' RACE. These cleaved targets had various functions based on their GO annotations. Interestingly, the expression of miRNAs and their targets were not negatively correlated.

In summary, these results showed the distinct expression of miRNAs in developmental seeds, and the cleaved targets of conserved miRNAs were also identified, both of which may lead to a better understanding of miRNAs in plant seed development.

Funding acknowledgement: National Transgenic Major Program of China (Grant No. 2011/2013ZX08003-002), National Basic Research Program of China (2012CB15301)

P113

Convergent Evolution of Maize phasiRNAs and Mammalian piRNAs Supporting Male Reproduction

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Animal germ cells specifically express PIWI-interacting RNAs (piRNAs) that contribute to transposon inactivation and germline development. While Drosophila piRNAs are mostly repeat-associated, mammalian piRNAs predominantly originate from unique genomic regions and are further classified as pre-pachytene or pachytene based on expression timing. Although highly abundant in most metozoans, the plant germline lacks piRNAs. Here we show that two novel classes of small RNAs (sRNAs) in maize anthers, the male reproductive organ of seed plants, peak at pre-meiotic and meiotic stages, respectively, and share characteristics with mammalian piRNAs. By sequencing sRNAs from ten sequential cohorts of developmentally staged anthers and mature pollen, we found that 21-nt phased siRNAs (21-phasiRNAs) from 463 loci emerge abruptly during pre-meiotic cell fate specification, while 24-nt phased siRNAs (24-phasiRNAs) from 176 loci coordinately accumulate as meiocytes mature and persist into mature pollen. Male-sterile mutants mac1 (arrests before metaphase I) and msca1 (lacks germ cells), fail to produce 24-phasiRNAs but have prolonged production of 21-phasiRNAs, while the partially male-sterile ocl4 mutant, defective in an epidermis-specific transcription factor, lacks 21-phasiRNAs but retains normal production of 24-phasiRNAs. By in situ hybridization, triggers of 21-phasiRNAs are found in the anther epidermis but 21-phasiRNAs are only abundant in inner layers. Both 24-phasiRNAs and their triggers accumulate strongly in the tapetum and meiocytes. While differences in biogenesis indicate their independent origins, grass phasiRNAs and mammalian piRNAs share characteristics such as developmental timing, lack of obvious target, and impact on male fertility. Our results suggest an evolutionary convergence of an essential role for sRNAs in male reproduction in advanced plants and animals.

Funding acknowledgement: National Science Foundation (NSF)

P114

Cytological Study and Fine Mapping of the Male Sterile Gene ms5 in Maize

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Male fertility in flowering plants relies on proper cell division and differentiation during anther development, a process that gives rise to four somatic layers surrounding central germinal cells. Many anther development deficient mutants have been isolated in maize, however, only limited number of male sterile genes have been cloned, and most of the cloned genes were involved in pre-meiotic process of anther development. The male sterility 5 (ms5) mutant is noteworthy because its male sterility is affected at microspore mitosis. Our cytological studies of ms5 showed that microspore genesis is normal through late-vacuolate microspore stage, Microspore wall and pore developments also were normal. Degeneration appeared to begin during the microspore mitosis stage. Generative-like and vegetative-like nuclei were observed in some degenerated microspores. Results of KI dyeing showed that starch deposition in pollen did not occur in ms5. ms5 was previously mapped to the long arm of chromosome 5. Based on the BC1F1 population (5000 individuals) from the cross between the male fertile line B73 and the male sterile ms5, ms5 gene was currently mapped between SSR markers P1-5 and P2-7, which spans 1.9Mb on the long arm of Chr.5. According to B73 sequences, there are 25 putative protein-coding genes in this region. Two of them are interesting in that they were expressed in pollen and involved in translational initiation according to their studies in Arabidopsis. Fine mapping and RNA-Seq analysis are under way to eventually clone the ms5 gene.

P115
Genetic Interaction between Bract Suppression Genes *tasselsheath1* and *tasselsheath2/4* in Maize

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The basic body plan the sporophyte of flowering plants consists of repeating units of internode and node, the latter of which contains one or more axillary meristem(s) and its subtending leaf. Different developmental mechanisms have evolved to suppress the outgrowth of either the axillary meristem or the subtending leaf, giving rise to modifications of the basic body plan. The suppression of subtending leaf (bract) in reproductive stage is especially common compared to the vegetative stage. In maize (*Zea mays*), several mutants, including *tassel sheath1* (*tsh1*), *tsh2/4*, *tsh3*, *tsh5*, and *Few branched1*, and multiple *enhancer of tsh1* mutants have been identified that affect bract outgrowth in both male (tassel) and female (ear) inflorescences, indicating a complex genetic network of bract suppression. To uncover the potential interactions of these genes, two best-characterized *tsh* loci that have strong effects on bract outgrowth have been chosen. Strong mutant alleles, *tsh1-ref* and *tsh2-ref* have been introgressed into B73 inbred background. An F2 population (consisting of 58 individuals) that segregates both single mutants and double mutants of both loci was generated. The phenotype of each individual in this F2 population was quantified by measuring tassel traits that are affected by *tsh1* and *tsh2/4*, including branch number, frequency of branch and spikelet pair bracts, frequency of solitary vs. paired spikelets, and length of bracts. These data will be analyzed for additive, epistatic, or synergistic interactions. qRT-PCR expression analysis of *tsh1* and *tsh2* transcripts in both mutant backgrounds, respectively, will be compared to look for evidence of a regulatory interactions among *tsh* loci.

Funding acknowledgement: National Science Foundation (NSF)

P116
Genetics and transcriptomics of bract suppression in maize

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After the transition from vegetative growth to reproductive growth, leaf development is suppressed in maize as it is in most other grasses. Leaves that grow after the reproductive transition are called bracts, and bract suppression evolved convergently in many angiosperm families including the grasses (Poaceae) and mustards (Brassicaceae). Previously we described two genes in maize necessary for bract suppression, *tassel sheath1* (*tsh1*) and *tsh4*. In order to identify other genes involved in bract suppression we have performed further screens for mutants that fail to suppress bract growth (*tsh** loci) as well as *enhancer of tsh1* (*ent**) loci. Multiple *tsh* and *ent* loci have been identified, indicating that bract suppression is genetically complex. Mapping of *tsh** and *ent** loci is currently underway, as is positional cloning of a dominant mutant *Few branched1* (*Fbr1*) that has reduced branching and ectopic bract growth in the tassel. In order to complement this forward genetics approach, we have profiled the transcriptome of *tsh1* tassel primordia using Illumina RNA-seq. Differentially regulated genes in *tsh1* mutants indicate that diverse hormone signaling pathways are regulated by *tsh1* to control bract growth. As a result of combining traditional forward genetics with transcript profiling, a complex network of genes and hormonal pathways required for bract suppression in maize is emerging.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P117

Lesion Mimics, Target Spot: Model for Cell Death Signalling

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The disease lesion (Les) mimics are the most frequently occurring dominant mutants from EMS mutagenesis in maize. From the screening of over 50,000 M1 plants for all variations of the lesion phenotype, we have identified 51 separate dominant cases. A much smaller, though probably comparable number of recessive les mutants, have been seen but not considered in this report.

The mutants range widely in expression, but have the common phenotype of leaf lesions that are strikingly similar to those caused by various leaf blight diseases. In all cases tested, the phenotypes have occurred in the absence of a pathogen. They are initiated by sunlight and certain chemicals, and can be chlorotic, necrotic, or sequentially both. The lesions of different mutants vary in size, shape, color, frequency, distribution, time of onset, position, rate of expansion, sharpness of boundaries, etc. In some mutants, the lesions expand to cover the leaf, resulting in senescence. It appears that particular cells on the leaf surface are, at specific developmental stages and within certain specifically variable temperature ranges, highly susceptible to damage by sunlight.

We hypothesize that two signals are involved. The first arises from dissolution of the cell membrane, which releases highly active cell contents that cause lethal damage to neighboring cells. This damage spreads continuously outward, forming a necrotic lesion that stops growing when conditions change. The second signal is revealed by the “Target Spot” oscillatory phenotype; a central spot of dead tissue surrounded by alternating rings of healthy and dead tissue. This phenotype suggests signaling between dead and living cells, across living tissue, that causes lesion formation. This signal may proceed more rapidly than the first, through several ranks of normal cells without damaging them during a diurnal cycle of conditions that do not favor lesion formation.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P118

Live cell imaging of the receptor like kinase WARTY2 and its role in epidermal patterning of bulliform-like cells during maize leaf development

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The maize leaf develops from the shoot apical meristem in a proximal to distal gradient of cell division, expansion and differentiation. The epidermis is a good model to study how the gradient is established because epidermal cells are arrayed in a predictable linear pattern and mutants with altered cell patterns are easily screened. We identified *warty2* (*wty2*) as a cell pattern mutant with overly expanded bulliform-like cells in the adaxial and abaxial epidermis. The WTY2 gene was cloned by a map-based method and shown to encode a novel receptor-like kinase (RLK) belonging to the LRR VII subfamily. Gene identity was confirmed with an additional EMS allele and two transposon insertions recovered from the UniformMu collection. Biochemical analysis showed WTY2/RLK was an inactive kinase. Three transgenic lines were produced, including a complementation construct, an over-expression line and a WTY2-YFP translation fusion. Stable transformants of these lines recovered the mutant wty2 phenotype, verifying gene identity and confirming the translational fusion was functionally tagged. Live cell imaging of the WTY2-YFP showed that the fluorescent protein (FP) fusion was localized to the plasma membrane as predicted by its transmembrane domain. The cellular localization pattern of WTY2-YFP depended on tissue type. In the leaf epidermis, the FP signal was localized in forming cell plates of dividing cells and also in the dynamic punctae within the cytoplasm. In all the tissues, the FP signal was not uniformly distributed in the plasma membrane. The WTY2-YFP signal in the epidermis was only observed in the plasma membrane adjacent to the inner periclinal cell wall and adjacent inner region of the anticlinal plasma membrane. In underlying mesophyll cells, the FP signal appeared preferentially localized to the plasma membrane in the orientation of recently divided cells. Our results implicate WTY2 as a new LRR-RLK involved in transduction of a positional signal that controls maize leaf epidermis differentiation.

Funding acknowledgement: National Science Foundation (NSF)

P119

Maize Cell Genomics: Developing a two component transactivation system

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Functional genomics tools are currently needed to leverage the quantity of sequence data being generated in maize. To provide resources for functional study, we have generated over 100 stable, natively expressed, fluorescent protein (FP) fusion lines that mark all common subcellular compartments in maize. These lines are publicly available and have been used by the maize research community for developmental, physiological and functional studies. We are currently developing an LhG4 two-component transactivation system to drive cell, tissue and organ-specific expression. Selected promoters activate expression of the LhG4 transcription factor, which in turn will transactivate genes of interest driven by the pOp promoter in responder lines. Currently, 22 driver constructs have been produced to drive expression in shoot and inflorescence meristems, leaves or roots using tissue-specific promoters. Four responder constructs are currently completed and being analyzed. Transformants have been obtained for 82% of driver constructs to date and expression analysis is underway. Recent advances and tests of the driver/responder system will be presented. The project will deliver to the research community 50 promoter/driver lines, 20 new FP tagged lines and will advance live cell imaging techniques using the resources generated. Seed availability, construct information and images are available at http://maize.jcvi.org/cellgenomics and at the JCVI Maize Bisque database, http://maize-bisque.jcvi.org. We encourage new requests for driver or responder lines from the maize community. Contact Dave Jackson or Anne Sylvester for if you have suggestions or need more information.

Funding acknowledgement: National Science Foundation (NSF)

P120

Mapping and Characterization of the Maize Mutant, *Clumped tassel1* (*Clt1*)

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During development from seed to mature plant, a delicate balance must be maintained between replenishing the undifferentiated cells in the meristems and consuming these meristem cells to form organs. In maize, overproliferation of meristems perpendicular to the growth axis often leads to "fasciation" in the inflorescences, forming flatter, broader ears and tassels. We are characterizing a semi-dominant mutant, *Clumped tassel1* (*Clt1*), which produces shortened, fasciated ears and tassels, with increased spikelet density in the tassel. In addition, leaf length and plant height are reduced. We have fine-mapped *Clt1* to a 0.53Mb region that contains 16 predicted genes. *Clt1* is an EMS allele in the non-reference background, ACR, and we are identifying the causative mutation by genome sequencing a line in which *Clt1* has been introgressed for ten generations into B73. Furthermore, additional *Clt1* alleles are being generated using *Ds* elements and EMS mutagenesis.

To further understand *Clt1* function, we have crossed the introgressed line with the NAM founder lines to identify naturally occurring genetic modifiers. In the F2 of a cross between *Clt1* (B73) and Ki11, we observe a novel *Clt1* phenotype in which the internodes in the upper plant are dramatically reduced. This enhancer segregates as a single, Mendelian recessive allele that we are mapping using bulked segregant analysis (BSA) on the Illumina Maize SNP50 chip, and validating using the B73 X Ki11 NAM recombinant inbred lines.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P121

Method development for an elite maize line callus transformation thru agrobacterium.

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A method using callus tissue derived from immature embryos of an elite maize line as initial explants to transform maize thru agrobacterium has been developed. We were able to generate multiple transgenic events using this procedure. The procedure involves harvesting immature embryos from maize ears and allowing them induce callus for 8-10 days. By which time the embryos grow into callus mass nearly 10 times its initial size. The calluses were then transformed using an appropriate agrobacterium strain with desirable selectable and reporter marker genes. During the process of transformation, the calluses were cut to 2-3mm size pieces thereby allowing more surface area and increase infection sites for agrobacterium. After infection the calluses were co cultivated in a suitable medium. The calluses were then transferred to a recovery medium containing an antibiotic to kill the agrobacterium on the callus tissue. The recovered calluses were then transferred to selection, regeneration and finally to rooting medium to yield transgenic events. The transgenic events were confirmed for the presence and expression of target genes thru molecular analyses and sent to greenhouse. The phenotype of T0 events obtained thru this method and their seed setting was found comparable to that of stock plants that were grown side by side from seeds. Some of the important applications for this method include, 1) less dependency of greenhouse grown stock material for immature embryos and 2) a quicker and more reliable screening of constructs for gene expression at callus stage.

P122
miRNA alterations are an important mechanism in the adaptation of maize to a low phosphate environment

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Maize is a globally important food and feed crop, and low phosphate supply in the soil frequently limits maize yields in many areas. MicroRNAs (miRNAs) play important roles in the development and adaptation to environment of plants. In this study, small RNAs from the root and leaf of the maize inbred line Q319 were subjected to low-phosphate culture conditions, analyzed with high-throughput sequencing technologies, and the changes in selected differentially expressed miRNA target genes were determined using real-time RT-PCR. Complex small RNA populations were detected after low-phosphate culture and displayed different patterns in the root and leaf. Among the detected miRNA families, miR159, miR166, miR167 and miR169 were highly expressed in the root, while miR156, miR164, miR172 and others demonstrated higher expression levels in the leaf. Some of the auxin-related miRNAs (miR393, miR160a/b/c, miR160d/e/g and miR167a/b/c/d) and their target genes showed co-operative changes in response to low-phosphate stress, and the downregulation of AtARF6/8-like genes in maize during low-phosphate stress could be major factors contributing to the lateral root reduction. Abiotic stress-related miRNAs engaged in interactions of different signaling and/or metabolism pathways. The changes in the expression of miRNAs and their target genes suggested that the miRNA alterations are an important mechanism in the adaptation of maize to a low phosphate environment, and some miRNAs participated in root architecture modification via the regulation of auxin signaling. A complex regulatory mechanism of miRNAs in response to the low phosphate environment exists in maize, which showed obvious differences from that in Arabidopsis.

Funding acknowledgement: Hi-Tech Research and Development (863 Program of China)

P123
Molecular Control of Grass Inflorescence Development

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The grass family is one of the largest families in angiosperms and has evolved a characteristic inflorescence morphology, with complex branches and specialized spikelets. The origin and development of the highly divergent inflorescence architecture in grasses have recently received much attention. Increasing evidence has revealed that numerous factors, such as transcription factors and plant hormones, play key roles in determining reproductive meristem fate and inflorescence patterning in grasses. Moreover, some molecular switches that have been implicated in specifying inflorescence shapes contribute significantly to grain yields in cereals. Here, we review key genetic and molecular switches recently identified from two model grass species, rice (*Oryza sativa*) and maize (*Zea mays*), that regulate inflorescence morphology specification, including meristem identity, meristem size and maintenance, initiation and outgrowth of axillary meristems, and organogenesis. Furthermore, we summarize emerging networks of genes and pathways in grass inflorescence morphogenesis and emphasize their evolutionary divergence in comparison with the model eudicot *Arabidopsis thaliana*. We also discuss the agricultural application of genes controlling grass inflorescence development.

P124

Ms23, a bHLH transcriptional factor, regulating the tapetal cell fate in pre-meiotic anther of maize

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A fully developed, fertile anther is composed of four wall layers, namely epidermis, endothecium, middle layer, and tapetum, surrounding the central pollen mother cells (PMCs). Anthers with tapetal defects are particularly known to cause male sterility. In *ms23* mutant anther, two defective "tapetal" layers are observed and the PMCs failed to progress beyond meiotic prophase I (Chaubal et al., 2000). We have successfully cloned *Ms23* (GRMZM2G021276), which is predicted to be a bHLH transcription factor. *Ms23* is highly expressed in young developing anthers compared to other tissues. Similar patterns have also been reported for the closest orthologs in rice and *Arabidopsis*. *Ms23* transcript is predominantly found in the tapetal layer based on the *in situ* hybridization result. Microarray data further identified genes, including a subset of small RNA biogenesis genes, which were differentially expressed in the *ms23* mutant anther. In comparison to the transcriptomes of other male sterile mutants, similar expression alteration is also observed, particularly in mutants with anther wall defects, indicating the significance of small RNAs in the development of fertile anthers.

Funding acknowledgement: National Science Foundation (NSF)

P125

Mutant analysis of maize antipodal cells and auxin signaling

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The female gametophytes of angiosperms have four cell types: the egg cell, synergids, central cell and antipodal cells arranged along the micropylar-chalazal axis. To explore the role of auxin in maize embryo sacs, expression patterns of two fluorescent reporters in maize (a reporter for auxin levels (DR5::RFP) and an auxin efflux carrier (PIN1a::PIN1a-YFP)) were examined. In mature maize embryo sacs both DR5 and PIN1a expression are expressed strongly and specifically in the antipodals. Embryo sac up-regulated genes from RNA-seq data were searched for genes related to auxin production and signaling. Genes required for all aspects of auxin production, distribution and signaling are represented in the embryo sac transcriptome. Preliminary in-situ's with Maize Auxin Response Factors (ZmARF's) support the conclusion that auxin is present and functional in maize antipodal cells. Maize mutants with reduced antipodal cell number lack both DR5 and PIN1a expression suggesting that auxin promotes antipodal cell proliferation in maize embryo sacs.

Funding acknowledgement: National Science Foundation (NSF)

P126
Preliminary Study of Polyembryonic Seedling in Maternal Haploid Induction

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Doubled Haploid (DH) has become one of the key technology of modern maize breeding, which also provide invaluable sources for maize genetic research. However, the mechanism of haploid induction still remains unclear. The phenomenon of polyembryonic seedling via haploid induction has been reported before, but few study has been done because of the low frequency. To better understand the rate and development of polyembryonic seedlings via maternal haploid induction, as well as the relationship with haploid induction, we chose 20 inbred lines and 10 single cross hybrids as female to cross with newly derived haploid inducers, part of which 68,178 kernels were harvested, and 3,244 haploid kernels were identified with the average rate of 4.76%. In addition, 56 kernels with poly embryos were also identified with the average frequency of 0.082% (56/68,178). The rate of polyembryonic kernels varied in different genetic sources (0~0.35%). This rate is significant higher compared to naturally occurring, but much lower than haploid kernel frequency demonstrating that haploid inducer can increase the frequency of polyembryonic kernel. In total, 182 polyembryonic kernels were obtained from all of the tested materials. All had purple color on endosperm inherited from inducer, and there were 152 with purple color on embryo, and 30 without purple color on embryo. Different shapes of germs among the twin were also observed. After germination, all the polyembryonic seedlings were twin seedlings, of which 8 pairs of haploid-haploid, 12 pairs of diploid-haploid, 122 pairs of diploid-diploid were survived, and 40 pairs were dead. Most of the twin seedlings has the same phenotypes during all the growth period. Part of the twin-seedlings had different phenotypes and the reason needs to be studied further.

Funding acknowledgement: This work was supported by National '863' High-Tech Program of China (Grant No: 2011AA10A103, 2012AA10A305) and Modern Maize Industry Technology System Foundation of China (Grant No: CARS-02-09)

P127
Quantifying maize tassel development and correlating tassel length with anther development

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In the study of early anther development, anther length is tightly correlated with developmental stages over the course of 8 days of development. But because the developing tassel is enveloped inside the whorl of leaves and is highly environmentally sensitive, determining the size of a young tassel without sacrificing the plant remains difficult. This can present problems when trying to study pre-meiotic anther development in planta. In this study a range of exterior characteristics (leaf number, stem circumference, and tassel height above soil line) were measured and correlated with tassel length. Anther lengths at 7 points on tassels were also measured to correlate tassel length with anther development at specific tassel locations. These results indicate that tassel length can occupy a wide-range for a given exterior measurement, but that anther development at specific tassel locations is highly reproducible for a tassel of known length. This information provides a useful tool for the study of anther development in the context of the maize tassel.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), American Society of Plant Biologists

P128

Regulation of maize kernel filling: the role of *ZmZOU* in embryo-endosperm communication

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In *Arabidopsis* the bHLH transcription factor AtZOU (ZHOUPI) has two functions in embryo-endosperm communication: it regulates (i) endosperm breakdown close to the growing embryo and (ii) the formation of a cuticle on the embryonic surface. In *atzou* mutants the seeds are characterised by a persistent endosperm reminiscent of the situation in maize. The maize genome contains a single gene *ZmZOU* in the same phylogenetic clade as *AtZOU*. The deduced amino acid sequence of ZmZOU has an N-terminal extension of 300 amino acids, which is conserved to increasing extent in rice and sorghum. The overall sequence similarity of 54% between AtZOU and ZmZOU increases to 86% in the conserved bHLH domain. *ZmZOU* expression is limited to the endosperm where it peaks during the filling stage. The phenotype of *ZmZOU*-RNAi lines is characterised by adhesion between embryo and endosperm, persistence of the suspensor and the embryo surrounding region. The analysis of RNAseq data from wild-type and transgenic seeds is in progress. The comparative analysis of the ZHOUPI transcription factor in maize and *Arabidopsis* provides a promising approach to understand the process of endosperm breakdown, which is conserved during early stages to make space for the growing embryo, but diverges in the final outcome in the mature seed.

Funding acknowledgement: Region Rhone-Alpes

P129

Teasing out the transcriptome of *in vivo* germinated pollen

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Growth of the pollen tube through the silk during pollination, which involves interaction between the male gametophyte and the female sporophyte, represents an important step in Zea mays reproduction. While advances have been made documenting the transcriptomes of mature pollen and silk, gene expression within the growing pollen tube is difficult to assess due to its location within sporophytic tissue. The approach presented here represents a novel use of RNA-seq technology to sort out the transcriptomes of the male gametophyte and the female sporophyte within a mixed RNA sample from pollinated silk by utilizing the large numbers of sequence polymorphisms (e.g. SNPs) present in maize. Polymorphisms identified by the HapMapv2 study (Gore et al. 2009, Chia et al. 2012), available through www.panzea.org, are inserted into the B73 reference genome to create “SNP genomes” for different inbred lines. Subsequently, comparison of the differential alignment of reads against the male and female parent genomes permits assignment of the subset of reads encompassing known SNPs to either the pollen or the silk. The advantage of this method is one can uncover the in vivo expression of pollen-specific and silk-specific genes, and thus identify genetic components potentially related to their interaction. Initial analysis of 23.6 million reads from a mixed sample of W22 silks and B73 pollen that align to chromosome 8 has detected expression of the B73 allele for 52 genes. These are genes whose expression putatively originates in the pollen tube. Consistent with this, 23 of these genes have been previously associated with expression enrichment in mature pollen. Validation by CAPS (Cleaved Amplified Polymorphic Sequence) analysis of RT-PCR products from independent silk/pollen RNA samples supports the detection of B73 expression in the mixed sample. Additional progress in extending the bioinformatic analysis to the entire genome will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P130

The *barren stalk2* Gene Is Required for Axillary Meristem Development in Maize

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The plasticity and diversity of plant architecture is determined by axillary meristem (AM) mediated lateral growth. AMs are small groups of stem cells produced in the axils of leaf primordia, which generate shoot branches (*eg*., maize tillers) and inflorescences (*eg*., maize ears and tassel branches). As maize yield depends on tassel and ear development, it is important to identify the genes and subsequent molecular mechanisms regulating AM formation. Previous studies identified several genes critical for AM production that function in auxin biosynthesis, transport or signaling. One of these genes is *barren stalk1* (*ba1*), which encodes a basic helix-loop-helix transcription factor acting downstream of auxin signaling to control AM formation. Although *ba1* is essential for AM production, it is not clear how *ba1* operates in a transcriptional network to regulate AM formation. We have identified a new mutant, *barren stalk2* (*ba2*), which, due to defects in reproductive AM formation, fails to produce ears, and has fewer tassel branches and spikelets, similar to the*ba1* mutant. Furthermore, the *ba2* mutation suppresses tiller growth in the *teosinte branched1* mutant, suggesting that it also plays an essential role in vegetative AM development. The *ba2* gene encodes a protein that co-localizes and heterodimerizes with BA1 in the nucleus. Characterization of the genetic interaction between *ba2* and *ba1* demonstrates that*ba1* is epistatic to *ba2* and shows a dosage effect in *ba2* mutants, providing further evidence that BA1 and BA2 act together in the same pathway. Characterization of the molecular and genetic interactions between *ba2* and other genes required for regulation of *ba1* further supports this hypothesis. We propose that heterodimerization of BA2 and BA1 is critical for AM formation and that these mutants provide an essential tool to dissect the gene regulatory network modulating AM production.

Funding acknowledgement: National Science Foundation (NSF)

P131

Types of morphogenesis in callus tissue of Lancaster maize group

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We have estimated the ability to various types of morphogenesis of maize calli derived from immature embryos of length in 1-1.5 mm. We have studied inbreds DK267, DK6080, DK420-1, DK298 and DK3070 of Lancaster germplasm, one of the most commercially and selectionally valuable for Ukraine, as well as model inbreds A188, PLS61 and Chi31.

Regenerated plants of all studied genotypes have been formed both through gemmogenesis and embryoidogenesis. However, embryoidogenesis significantly prevailed in model lines (77,61 ± 3,64%), while both types of morphogenesis were found with the same frequency in Lancaster inbreds (57,69 ± 11,26 and 42,31 ± 11,26%, respectively). Gemmogenesis prevailed over the embryoidogenesis in hybrids, which included the model inbred PLS61 (86,63 ± 4,99%). The influence of the duration of cultivation on callus induction medium on the type of morphogenesis was observed for hybrids with the participation of A188 and Chi31. Gemmogenesis was observed more frequently when thirty-day calli had been transplanted on the regeneration medium (93,67 ± 5,51% for hybrids with A188 and 80,00 ± 26,67% for hybrids with Chi31). When sixty-day calli of hybrids with A188 had been transplanted on the same medium the embryogenesis prevailed (70,59 ± 22,78%). Levels of gemmogenesis and embryoidogenesis did not significantly differ for hybrids with Chi31 (57,89 ± 23,27 and 42,11 ± 23 , 27%). Increasing the duration of cultivation on the callus induction medium led to the formation of additional embryos.

Thus, the type morphogenesis in maize culture in vitro depends strongly on the genotype of an explant, as well as the duration of cultivation of calli. To increase the yield of regenerated plants it can be recommended to use Lancaster inbreds and their hybrids with model lines A188 and Chi31, as well as to prolong the period of cultivation of calli until transfer to the regeneration medium.

Funding acknowledgement: National academy of agrarian sciences of Ukraine

P132

Water acts as a positional signal to pattern root architecture in maize

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The architecture of the branched root system is a major determinant of plant vigor. Water availability is known to impact root physiology and growth however the spatial scale at which this stimulus influences root architecture is poorly understood. Here we reveal that differences in the availability of moisture across the circumferential axis of the root create spatial cues that determine the position of lateral root branches (LRs). We show that roots of several plant species including maize can distinguish between a wet surface and air environments, and that this also impacts the patterning of root hairs, anthocyanins, and aerenchyma in a phenomenon we describe as hydropatterning. This environmental response is distinct from a touch response and requires available moisture to induce lateral roots along a contacted surface. MicroCT X-ray tomography and 3-D reconstruction of soil-grown root systems demonstrated that such responses also occur under physiologically relevant conditions. Hydropatterning is independent of endogenous ABA and ethylene signaling, distinguishing it from a classic water-stress response. This work reveals that local environmental stimuli impacts lateral root patterning consistent with the spatial scale such stimuli vary with in a natural soil context.

Funding acknowledgement: National Science Foundation (NSF)

P133

Zm908p11, encoded by a short open reading frame (sORF) gene, functions in pollen tube growth as a profilin ligand in maize

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Double fertilization of flowering plants depends on the targeted transportation of sperm to the embryo sac by the pollen tube. Currently, little is known about the underlying molecular mechanisms that regulate pollen germination and pollen tube growth in maize (*Zea mays*). Here, a maize pollen-predominant gene *Zm908*, with several putative short open reading frames (sORFs), was isolated and characterized. The longest ORF of *Zm908* encodes a small protein of 97 amino acids. This was designated as Zm908p11 and is distributed throughout the maize pollen tube. Western blot detected the small peptide in mature pollen. Quantitative reverse transcription–PCR and northern blot analysis revealed that *Zm908p11* was expressed predominantly in mature pollen grains. Ectopic overexpression of full-length *Zm908* and *Zm908p11* in tobacco resulted in defective pollen, while transgenic tobacco plants with a site-specific mutation or a frameshift mutation of *Zm908p11* showed normal pollen development. Overexpression of *Zm908p11* in maize decreased pollen germination efficiency. Maize pollen cDNA library screening and protein–protein interaction assays demonstrated that Zm908p11 interacts with maize profilin 1 (ZmPRO1). A microarray analysis identified 273 up-regulated and 203 down-regulated genes in the overexpressing transgenic *Zm908p11*pollen. Taken together, these results indicate that *Zm908* functions as Zm908p11, and binds to profilins as a novel ligand, with a required role during pollen tube growth in maize. Accordingly, a model is proposed for the role of Zm908p11 during pollen tube growth in maize.

Funding acknowledgement: Natural Science Foundation ofChina (grant no. 30971555) and the National Basic Research Program of China (2012CB215301).

P134

ZmLAZY1 regulates shoot gravitropism and inflorescence development through polar auxin transport and auxin signaling

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Auxin transport has been believed to be responsible for tropism and morphogenesis for over a century. Maize has distinct male (tassel) and female (ear) inflorescence, and this morphogenesis depends on auxin maximum and gradient. The classic maize mutant *lazy plant1* (*la1*) has defective gravitropic response, while the mechanism underlining maize gravitropism remains largely elusive. Here we report the maize *prostrate* (*ps1*) mutant, which is allelic to *la1*, displays prostrate growth with reduced shoot gravitropism and defective inflorescence development. Map-based cloning identified maize *ZmLA1* as the functional ortholog of *LAZY1* in rice and in Arabidopsis, and with unique role in inflorescence development and enriched expression in reproductive organs such as tassel and ear. Transcription of *ZmLA1* is responsive to auxin and repressed by light. Further, ZmLA1 physically interacts with a putative auxin transport regulator in the plasma membrane and a putative auxin signaling protein in the nucleus. RNA-SEQ data showed that dozens of putative auxin transport, auxin response and light signaling genes were differentially expressed in the *la1* mutant stems. Therefore, we propose a model describing the ZmLA1-mediated complex interactions among auxin, gravity, light and inflorescent development.

Funding acknowledgement: Ministry of Science and Technology, Natural Science Foundation of China, National Transgenic Key Project

P135

A novel central element of the synaptonemal complex is required for centromere pairing in maize

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In most eukaryote species, segregation of homologous chromosomes during meiosis is established through homologous chromosome pairing, synapsis, and recombination. The underlying mechanism behind homologous chromosome pairing is poorly understood, we found that centromere pairing begins at the leptotene stage and is earlier than the formation of telomere bouquet in maize. This observation indicates that centromere pairing may play an important role in homologous chromosome pairing initialization. In several species, homologs of the ZIP1 protein, which forms the central element of the synaptonemal complex in budding yeast, play essential roles in centromere coupling. However, we found that in maize, centromeres form associations before the maize ZIP1 homolog installs in the centromeric regions of chromosomes, which suggested that ZIP1 may not participate in establishing early prophase centromere pairing. We hypothesized that another, yet uncharacterized SC component could control centromere pairing instead of ZIP1. Through phylogeny reconstructions, we found that the SMC (Structural Maintenance of Chromosomes) domain of ZIP1 is homologous to the SMC domain of SMC6, a subunit of the SMC5/6 complex. Using the sequence of the rice *SMC6* gene, we conducted a BLAST search to find a maize *SMC6* homolog. Immunolocalization pattern showed that ZmSMC6 colocalized with ZIP1, which indicated that SMC6 is a novel component of the central element of maize synaptonemal complex. Results from *SMC6 RNAi* lines revealed that, in contrast to the wild-type, centromeres were not paired from leptotene to pachytene stage. These data suggest that ZmSMC6 most likely plays a role in facilitating centromere pairing similar to this played by ZIP1 homologs in other species.

Funding acknowledgement: National Basic Research Program of China (973 Program 2011CB944601)

P136
Bub1 and Bub3 Play An Important Role in Plant Chromosome Orientation and Separation

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The genomic stability of all organisms requires a precise cell division both in mitosis and meiosis, and the successful partitioning of chromosomes to daughter cells relies on the proper orientation. Meiosis II resembles mitosis in using bi-orientation but employs a unique mono-orientation in meiosis I. The key factors associated with the chromosome orientation and segregation, such as Monopolin Complex and Spindle assembly checkpoint (SAC) components have been identified in yeast and mammalian cells. The SAC kinase Bub1-mediated histone H2A-S121 phosphorylation, cooperates with Haspin-mediated histone H3 threonine 3 (H3-pT3) phosphorylation to recruit Aurora B to the inner centromeric region. Bub3 is also an important factor that facilitates cell division through metaphase to anaphase, and its localization is dependent on Bub1. Our previous work has found that several maize minichromosomes exhibit bi-orientation in meiosis I, which is quite different from the normal mono-orientation. In order to study the mechanism of chromosome orientation and segregation in maize minichromosomes, we cloned the homologous genes of bub1 and bub3 in maize. Immunostaining indicates that Bub1 localizes in the centromeric regions in both somatic cells and meiocytes in a cell-cycle dependent process, and Bub3 has a similar localization like Bub1. Bub1 RNAi transgenic plants showed a dramatic decrease in the level of histone H2A phosphorylation. Comprehensive analysis of these RNAi transgenic plants and minichromosomes will shed light on the role of Bub1 and Bub3 in chromosome orientation and segregation in plant meiosis.

Funding acknowledgement: National Science Foundation (NSF), the National Basic Research Program of China (973 Program)

P137
Centromere changes in progeny of misdivision

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In maize, many small chromosomes can originate from the process of misdivision or breakage-fusion-bridge (BFB) cycle. In one reciprocal translocation material TB-9Sb, the chromosome B-9 with an active B centromere, conducts misdivision during meiosis, which produces different derivatives such as iso, telo and ring chromosomes. Most of these derivatives produce new variants in the following generations, which can be seen in changes of chromosome construction and centromere location. Wondering why so many variants can be formed during misdivision, we focus our work on centromere variation of derivatives of TB-9Sb. We detected centromeric location and centromeric DNA sequences in the offspring of TB-9Sb via fluorescence in situ hybridization and found that centromere specific sequences can be reduced or eliminated. During the reactivation process of an inactive centromere in 9-Bic-1, we found several stable new chromosomes formed with changed centromeres. In the irradiation of trisomic lines of maize, we also recover the centromere changes from the hybrids by using the gama ray treated the pollen of trisomic lines. So far, we find that centromere variation is one of the important phenomenon in misdivision derivatives, which may have function in the process of misdivision.

Funding acknowledgement: National Science Foundation (NSF)

P138

Differential gene expression of maize (Zea mays L.) inbred line H99 during somatic embryogenesis

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Abstract: Somatic embryogenesis is a complex developmental process that offers great potential in plant propagation. the mechanism of onset of somatic embryogenesis is not well understood. cDNA-amplified fragment length polymorphism (cDNA-AFLP) analysis was used to evaluate gene expression in embryonic and non-embryonic callus of inbred line H99 during the process of embryogenesis. A total of 101 candidate genes associated with the formation of maize embryonic callus were identified. Based on the sequence analysis, a total of 53 TDFs of known functions were involved in energy produce and conversion, cell division and signal transduction etc, suggesting that somatic embryogenesis undergoes a complex process. Two full length cDNA, encoding KHCP (kinesin heavy chain like protein), TypA (Tyrosine phosphorylation protein A) and partial sequences of encoding ARF-GEP (guanine-nucleotide-exchange protein of ADP ribosylation factors) homologues were isolated from embryonic callus of maize and named as ZmKHCP, ZmTypA and ZmARF-GEP, respectively. Finally, the expression levels of the three genes showed a significant increase between embryonic callus and non-embryonic callus as analysed by real-time qRT-PCR. As a consequence, this study provides important clues to understanding the induction of somatic embryogenesis in maize, and the candidate genes associated with the formation of embryonic callus might offer additional insights into the mechanism of the somatic embryogenesis formation and the three candidate genes need further research to determine their usefulness in breeding high induction rate of embryonic callus cultivars for transgenic breeding.

P139

Identification and Characterization of ZmHaspin kinase as an essential kinase during cell division

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Haspin kinases are members of a divergent group of the eukaryotic protein kinase family that are conserved in many species including fungi, plants and animals. As the kinase phosphorylates H3 at Thr3 in vitro and in vivo, haspin can be found in all eukaryotes and most of them only have one copy except budding yeast and C .elegans. Previous research has shown that Haspin localizes to mitotic chromosomes to phosphorylate histone H3 at Thr3, and combines with the phosphorylated histone H2A at Thr121 (fission yeast) catalyzed by kinase Bub1, leading to the recruitment of proteins responsible for proper chromosome segregation. During cell division, accurate chromosome orientation and segregation is necessary for cell fate. In order to study the mechanism of chromosome orientation and separation, we cloned the homologous gene for haspin in maize. By Blast and alignment, one can see that ZmHaspin kinase shows great similarity with other Haspin kinases. The phylogenetic analysis also confirms that Haspin kinases are conserved among different species, which might be related to their conserved function. We also found that the haspin gene in maize has two different transcripts, both of which can be amplified in different tissue samples especially with a high level of expression. At the same time, we detected the expression pattern in afd1 mutants; the results indicate that haspin gene is down-regulated severely because of Rec8 deletion, which is in accord with the function of haspin through the cohesion complex. Immunostaining demonstrates that ZmHaspin localizes along the chromosome arms during pachytene stage in meiocytes. Further detailed analysis of the RNAi transgenic plants will provide new insights to the function of ZmHaspin in chromosome orientation and segregation during cell division.

Funding acknowledgement: National Science Foundation (NSF), National Basic Research Program of China

P140
Isolation and Flow Purification of Endosperm Protoplast in Developing Seed of Maize

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The endosperm of maize grain is the main place for synthesizing starch and the developmental progression of the endosperm cell is the predominant factor for building of grain. Separation of endosperm cell protoplasts will be able to offer the homogeneous experimental materials for in vitro culture of endosperm cell, transcriptome analysis and typing of endosperm cell which is helpful for understanding of molecular mechanism of seed development. On the basis of previous studies, this paper optimized a separation method of maize endosperm protoplast isolation with adjusting the combination and concentration of enzyme as well as the utilization of the membrane stabilizer and osmotic stabilizer. The protoplasts were further purified by flow sorting. Our results showed that a lot of crude protoplast could be obtained when endosperm tissue was digested in MS medium containing 0.5% macerozyme, 0.5% hemicellulase, 1% cellulose, 0.7–0.8 mol L-1 of osmotic stabilizer and 0.8 mol L-1 of membrane stabilizer for 4 h at 30℃. The more than 90% of purified protoplasts would maintain viability when protoplasts stained by Fluorescein diacetate (FDA) were checked under a fluorescent microscope. The viable protoplasts could be accumulated and purified from the crude protoplast suspension by flow sorting technology.

P141
Problem of seed fertility of tetraploid corn and possible ways of its decision

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The autotetraploid corn is received for the first time in the USA in 1931 by L.F. Randolf by method of temperature shock. Existence of more than two allelic in one locus of autotetraploid corn considerably expands possibilities of their interactions. It can lead to increase heterosis. Therefore value of selection and genetic studying of tetraploid forms of corn actually.

Early works of many researchers showed prospects selections of autotetraploid corn, but low seed efficiency of the first autotetraploids didn't allow to create commercial hybrids quickly. This fact sharply reduced enthusiasm of researchers. Opinions of scientists, on the reasons causing low seed fertility autotetraploids, differ. The classical concept about the reasons of fall of seed efficiency is based on data of research of cytology meiosis at autotetraploids. The analysis showed multivalent association of homologous chromosomes. As a result of it in meiosis at autotetraploids, there are gametes with an unbalanced set of chromosomes which became the reason of decrease in seed efficiency owing to emergence aneuploids. The similar situation repeated on experimental autotetraploids other grain crops. Determination of specific weight of the cytogenetic reasons in decrease in seed efficiency autotetraploids, and also development of effective methods of its increase continues to remain an important problem of genetic and selection researches of this culture.

The author developed a method of receiving highly productive genotypes of tetraploid corn on the basis of complex studying and selection in population of genotypes by certain morphological and cytological criteria. On the basis of a selection material received as a result of the conducted researches the new grade of tetraploid corn included in the register of selection achievements of the Russian Federation was created.

Funding acknowledgement: United States Department of Agriculture (USDA)

P142
The Regeneration System of a High Oil Maize (GY302)

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Maize has only a few of regeneration-competent genotypes in tissue culture, Hence, enlarging the resource is pivotal importance to both basic and practical application for regeneration and transformation. Here, taking A188 as the control, we developed an efficient tissue culture and plant regeneration system of immature embryos of high oil maize (GY302). The influences of genotype, medium and AgNO3 content were investigated, the embryogenic calli ratios of GY302 and A188 were 67.03% and 51.24%, respectively. N6 medium was the optimal media for the development and long-term subculture of GY302 embryogenic calli. In the stage of regeneration, the MS high glucose medium was the most suitable condition for the formation of abundant embryoids and the rate of seedling success was as high as 65.38%. The results of this study would optimize the in vitro culture and regeneration system from maize immature embryos, especially the high oil maize, amplify the cereal materials that could produce regeneration-competent embryogenic calli and then could be used as the recipients of interesting exogenous gene in genetic engineer.

Funding acknowledgement: Project of Major Special Subject of Breeding New Transgenic Varieties (2013ZX08003-002), National '863' High-Tech Program of China (Grant No: 2011AA10A103, 2012AA10A305)

P143
Chinese Translation of MaizeGDB and the Maize Genetics Conference websites

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Beijing, China's selection as the venue for the 2014 Maize Genetics Conference (Maize Meeting) was due in large part to China's increased investment and interest in agricultural research (which heavily includes research in maize genetics and genomics). The China-hosted conference is anticipated to foster and advance new and existing collaborations among maize researchers worldwide. To facilitate this effort, MaizeGDB, the maize research community's centralized, long-term repository for genetic and genomic information (http://www.maizegdb.org), formed a collaboration with Chinese colleagues to manually translate entry-level pages of the MaizeGDB and Maize Meeting websites. This effort to make both the conference website and MaizeGDB more accessible to Chinese-speaking researchers not only makes sense given the 2014 meeting venue, it also makes sense as evidenced by MaizeGDB site usage statistics. Over the past five years the percent of visitors to MaizeGDB from China has increased from 19% to 31% and the percent of users who report Chinese as their primary language has risen to 30%. What's more, each year about 20% of the usage from China is from new users. Primary goals of the translated websites are to provide improved ease-of-use for new and existing Chinese users and to help facilitate international data sharing and collaboration.

Funding acknowledgement: United States Department of Agriculture (USDA)

P144
Education and Outreach at MaizeGDB

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At MaizeGDB, we fully appreciate that researchers want to be able to use online tools with a minimum amount of time spent learning how to use the tools. To provide help, we are creating a series of short online tutorial videos that aim to quickly show researchers how to access specific data and tools using the new interface. Each video is under 3 minutes, and has a descriptive title to guide researchers to the tutorial that best addresses their needs. Videos on a single topic are grouped together so that you can quickly find the precise topic you want to learn more about. In addition, we are expanding the written descriptions and explanations under all the questions marks "?" on each page. We are striving to provide more helpful "how-to" and "how this works" content on all relevant pages. As always, we greatly appreciate your feedback and we invite your comments and suggestions.

Funding acknowledgement: United States Department of Agriculture (USDA), National Corn Growers Association

P145
Evolution of the Maize Research Community

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MaizeGDB is a database of maize genetics and genomic information accessible world wide. Researchers use MaizeGDB regularly for biological investigations, but we‘re leveraging it in a different way: to better understand the evolution of the research community. Data mining reveals details such as distribution of maize cooperators by country and, within the US, by state. By analyzing the collection of articles curated into MaizeGDB, we've determined which words in are the most prevalent, which authors are listed on the most publications, and much more. Stop by to learn some lesser known facts about the maize research community!

Funding acknowledgement: United States Department of Agriculture (USDA)

P146

New avenues to contribute your data to MaizeGDB: *gene functions, genome assembly problems, maize gene wiki*

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As a community database, MaizeGDB strives to find new ways for you to contribute your data, both as free text, and in ways that support computable standards, such as Gene Ontology annotations. This allows MaizeGDB to capture more of your collective expertise. One route for researchers to enter their own data is to link your publications to a gene or gene model. This tool is listed under the Annotations section on the gene/model page, on the new MaizeGDB website (http://alpha.maizegdb.org). It links to a web services portal, http://bioportal.bioontology.org/, so that researchers may access the most current versions of the standard dictionaries for functional annotations. Currently MaizeGDB supports the Gene Ontology, Plant Ontology and Trait Ontology controlled vocabularies. The tool provides credit to the person providing the information. Gene Ontology data are reviewed and forwarded to metabolism databases (CornCyc, MaizeCyc, PlantCyc, MetaCyc) and UniProt. A second tool collects documented information about gene model or genome assembly problems. For example, the current B73 reference genome assembly is missing several of the repeated copies of the *p1* gene described by Goettel and Messing (2013) Theor Appl Genet 126:159-177. The goal of this tool is to aid in stewardship of the reference genome assembly, in collaboration with sequencing teams and the NCBI.

We have installed a community accessible wiki 'maize gene reviews' http://maizegenereview.maizegdb.org/. As with the standard wikipedia, the site is completely open, using standard practices to avoid irrelevant contributions. We invite participants at this meeting to add their own pages and/or to update any existing pages. MaizeGDB staff attending the meeting will be happy to assist. Articles may also be linked to MG Neuffer's recent Maize Phenotype wiki, mutants.maizegdb.org. MaizeGDB gene pages will link to this community wiki.

As in the past, persons with very large datasets are encouraged to contact MaizeGDB prior to submitting grant applications.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), National Corn Growers Association (NCGA)

P147

DNA Subway: A Simple, Powerful Bioinformatics Workflow for RNA-Seq Analysis and Distributed Genome Annotation

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This session introduces DNA Subway (dnasubway.iplantcollaborative.org), an educational bioinformatics platform developed by the NSF-sponsored iPlant Collaborative. DNA Subway bundles research-grade bioinformatics tools and databases into intuitive workflows - presenting them in an appealing interface modeled on the metaphor of a subway map. “Riding” on different DNA Subway lines, students can predict and annotate genes in up to 150 kb of DNA (Red Line), identify homologs in sequenced genomes (Yellow Line), and analyze DNA barcodes and construct phylogenetic trees (Blue Line). The new Green Line provides an easy-to-use workflow to analyze RNA-Seq experiments, the most accessible whole genome datasets. Based on the popular Tuxedo Protocol, the RNA-Seq workflow uses high performance compute resources of NSF’s Extreme Scientific and Engineering Discovery Environment (XSEDE) – providing the first readily available "on ramp" to biological supercomputing. Locally generated RNA-Seq data is seamlessly transferred from the Green Line to a newly upgraded Red Line, which incorporates WebApollo and JBrowse The annotation workflow readily accepts any type of GFF file – including output from Maker and evidence from other genome resources. DNA Subway’s integrated analysis, annotation, browser system creates a “power desktop” allowing students to explore large-scale variation in gene expression and genome structure on their personal computers. We are specifically adapting this system to support distributed annotation of the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

P148

The CIMMYT Maize Lines (CMLs): A critical genetic resource for maize breeders and geneticists

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The CIMMYT Maize Lines (“CMLs”) are a set of over 500 inbred lines, developed over the past 60 years by CIMMYT breeders throughout the world. In 2005, the conservation, regeneration and distribution of this important genetic resource became the responsibility of the CIMMYT Maize Germplasm Bank (MGB). Prior to this, the breeders who developed the lines were responsible for their maintenance. In 2013, the MGB and collaborators in the MasAgro Biodiversity Initiative (“Seeds of Discovery”) started a project to genetically compare the original germplasm received in 2005 with the current germplasm being distributed, checking for any evidence of drift or contamination. The ultimate goal is to produce a standard genetic fingerprint for each CML that will be used in routine quality control testing after every regeneration. Here we present the preliminary results from this analysis.

Funding acknowledgement: MasAgro (Government of Mexico), CGIAR Genebank Research Program

P149

The Maize Germplasm Bank at CIMMYT: Global source of maize genetic resources

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Every accounting of the current status of maize genetic resources places CIMMYT's Maize Germplasm Bank (MGB) in the number one position: it is the largest global collection of maize germplasm in the world, numbering over 27,000 accessions. However, this represents only nine percent of the world's *ex situ* holdings, not accounting for duplicates. The global representation of the maize accessions in the MGB reflects the New World, and specifically, Mexican, origin of this crop plant, with 97% of its collection originating from Mexico, South America or Central America. Temperate maize germplasm is well-represented in other germplasm banks, such as the USDA-ARS North Central Regional Plant Introduction Station (Ames, Iowa, USA) and the Institute of Crop Germplasm Resources at the Chinese Academy of Agricultural Sciences (Beijing, China), the second and third largest collections of maize germplasm in the world. Most of the collection (87%) is unimproved landraces, but there are some important improved materials, primarily generated by CIMMYT breeders. This includes the CIMMYT Maize Lines (CMLs), 540 inbreds that are used for breeding and genetics studies all over the world. MGB's crop wild relatives collections include: the teosintles, 270 accessions of *Zea* that are not domesticated maize, and 160 accessions of *Tripsacum*, the sister genus to *Zea*. The teosintle germplasm is seed, and is stored as is maize. *Tripsacum* accessions are maintained as live plants. In addition to the routine activities to support the conservation, regeneration, characterization and distribution of the collection, the MGB actively participates in research with various collaborators, including: the monitoring of the unintentional presence of transgenes in MGB regeneration nurseries, using sentinel rows and pollen traps; the study of various methods for the control of ear rot in maize landraces; the establishment of genetic fingerprints for CIMMYT Maize Lines (CMLs).

Funding acknowledgement: Global Crop Diversity Trust, CGIAR Genebank Research Program

P150

The Over view of a Typical Maize waste products usage in Gwagalada Area Council of the Federal Capital Territory.

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The disposal of waste product in Maize have a lot of different methodology .The manners in which the cobs ,leaves and stems are being disposed have being a serious problems in some farm visited in Gwagalada area council of the Federal Capital Territory , Abuja Nigeria .
A household was visited in Old Compensation Layout of Gwagalada in which. A woman farmer make use of the Maize cob in the drying of fish for commercial purposes . Other farms were visited. Questionnaires were distributed, and pictures were taken. This paper present the different findings and How International countries can contribute to the enhancement of the Maize cob Technology in Fish.

Funding acknowledgement: Michael Adedotun Oke Foundation /Individual

P151

A molecular chaperone ZmTRXh confers resistance to sugarcane mosaic virus (SCMV) in maize

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Sugarcane mosaic virus (SCMV) causes the devastating viral disease in maize and poses a grave threat to maize production worldwide. Two major genes, *Scmv1* which shows strong early resistance to SCMV and *Scmv2* that mainly functions at later stage, are required to confer complete resistance to SCMV. We located the *Scmv1* locus into a 59.21-kb region by integrating data from multiple mapping populations. We then used the *Scmv1*-tagged markers to screen three BAC libraries constructed from the resistant lines, FAP1360A, Huangzao4, and 1145, and the resultant positive BAC clones were assembled into three separate resistant BAC contigs for sequencing and gene annotation. Comparison of three resistant inbred lines to the reference B73 (susceptible to SCMV) for the predicted genes or ORFs within the 59.21-kb *Scmv1* region pinpoints a single candidate gene *ZmTRXh*, which encodes an h-type thioredoxin (TRXh) and shares the identical coding sequence among the resistant and susceptible inbred lines. The expression levels of *ZmTRXh*varied dramatically among different tissues, and the highest expression level was detected in mature leaf tissue. Moreover, the expression level of *ZmTRXh* in leaf tissue was significantly higher in resistant inbred lines than susceptible lines. Transgenic lines with the exogenous *ZmTRXh* gene from the resistant line 1145 showed enhanced resistance percentage by 30~40% at 7 days post inoculation. Over-expression of *ZmTRXh* in maize protoplasts could repress the propagation of SCMV. The ZmTRXh protein contains atypical active sites, which failed to reduce disulfide bridges but exhibited the chaperone activity. It could be concluded that the h-type thioredoxin maybe acts as a molecular chaperone to protect R gene to confer resistance to SCMV.

Funding acknowledgement: National High-tech and Development Program of China

P152

A Novel Maize Homeodomain-leucine Zipper (HD-Zip) I Gene, Zmhdz10, Positively Regulates Drought and Salt tolerance in both Rice and Arabidopsis

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Increasing evidence suggests that HD-Zip I transcription factors play important roles in abiotic stress responses, but no HD-Zip I proteins have been reported in maize. In our previous study, 17 HD-Zip I proteins were identified in the maize genome and divided into eight subgroups based on phylogeny. As a continuation of the previous study, this work focused on the isolation and functional characterization of one member, Zmhdz10, a drought-induced HD-Zip I gene. RT-qPCR showed that expression of Zmhdz10 was also induced by salt stress and abscisic acid (ABA). Transient expression of Zmhdz10-GFP fusion proteins in onion cells showed a nuclear localization of Zmhdz10. Yeast hybrid assays demonstrated that Zmhdz10 has transactivation and DNA-binding activity in yeast cells. Overexpression of Zmhdz10 in rice led to enhanced tolerance to drought and salt stresses and increased sensitivity to ABA. Moreover, Zmhdz10 transgenic plants had lower relative electrolyte leakage (REL), lower malondialdehyde (MDA) and increased proline (Pro) content relative to WT plants under stress conditions, which may contribute to enhanced stress tolerance. Zmhdz10 transgenic Arabidopsis plants also exhibited enhanced tolerance to drought and salt stresses that was concomitant with altered expression of stress/ABA-responsive genes, including Δ1-Pyrroline-5-carboxylate synthetase 1 (P5CS1), Responsive to dehydration 22 (RD22), Responsive to dehydration 29B (RD29B) and ABA-insensitive 1 (ABI1). Taken together, these results suggest that Zmhdz10 functions as a transcriptional regulator that can positively regulate drought and salt tolerance in plants through an ABA-dependent signaling pathway.

Funding acknowledgement: National Science Foundation (NSF)

P153

A simple, novel and high efficiency transformation method to introduce foreign DNA into corn plants.

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Genetic engineering breeding and identification of novel genes in maize are very dependent on high efficiency of corn transformation. The major deficiencies in current plant transformation systems include but are not limited to the production efficiency of the system, transformation variability due to genotype or species diversity and explant limitations and a long labor-intensive process requiring much skill. In particular, there is a continuing need in the field of plant biotechnology to provide more efficient, simple and low cost transformation methods suitable for high capacity production of economically important plants, particularly elite cultivars. Cell-penetrating peptides (CPPs) were discovered to protect DNA from degradation and deliver a varity of moleculars including DNA into cell. Here, we studied the function of CPPs （Tat2） in improving maize transgenic efficiency through pollen tube method. The transformation experiments in Jing 24 and 178 corn inbred lines were conducted under the different conditions of linear DNA concentration, ratio of Tat2, calcium, sucrose, ATP, and GTP concentration. Screened with herbicide for T0 seedlings, the optimal transformation system was obtained by the mediation of Tat2 through pollen tube transformation method, its tansgenic efficiency was up to 1-1.5%. Screened with herbicide for T1 seedlings, genetic inheritance and segregation of T1 progeny was revealed under this genetic manipulation, supporting a genetic ratio 3:1 of T1 progeny was about 16-18% in all transgenic lines. This is a novel transgenic method for corn with high efficiency, safe, low cost and no limitation of corn genotype.

Key words: cell penetrating peptide; novel transgenic method; pollen tube method; high transgenic efficiency for corn

Funding acknowledgement: Foundation of Beijing Municipal Committee of Science & Technology, National Special Program of Transgenic Research

P154

A Systems Approach to Identify Genetic Control of Tocochromanol Variation in Maize Grain

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The identification and characterization of genetic factors controlling variation for tocochromanol composition and concentration in maize grain has major health and nutritional ramifications for substantially increasing the availability of vitamin E and antioxidants in plant-derived foods. To date, our joint linkage-genome wide association study (JL-GWAS) of six tocochromanol compounds and 14 of their sums, ratios, and proportions in the nested association mapping (NAM) panel has provided unprecedented insight into the genetic control of tocochromanol synthesis in maize grain. Specifically, grain tocochromanol composition and concentration appear to be highly heritable, with additive models identifying between 5 and 22 QTL for each of the evaluated traits. Our tests for pleiotropy indicated differential degrees of shared QTL between the six tocochromanol compounds, with 90% of QTL being shared between two compounds exhibiting over two-fold differences in vitamin E activity. Although the strongest signals in our analysis coincided with tocochromanol biosynthetic pathway genes such as *ZmVTE4*, *ZmVTE1*, and *ZmHGGT1*, two-thirds of the identified QTL did not co-localize with any of 60 *a priori* candidate biosynthetic genes. To help further characterize the genes that underlie these QTL, we queried RNA-Seq data derived from six stages of kernel development across the NAM founder lines. Using these data, we confirmed that differential expression of *ZmVTE4* and *ZmHGGT1* account for variation in tocochromanol levels. Co-expression analyses summarizing the patterns of correlation between global gene expression and tocochromanol profiles were also explored. The contribution of differential expression to tocochromanol variation is also being evaluated by examining the proportion of GWAS signals located within regulatory regions. Ultimately, this work constitutes the most comprehensive dissection of tocochromanol variation in maize grain, and the candidate genes identified both within and outside of the tocochromanol biosynthetic pathway will provide new target loci for vitamin E biofortification programs.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P155

Advancing the production of maize haploids in-vivo

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Creation of high-efficient inducers of maternal haploids in the past 10-20 years enabled broad implementation of doubled haploid technology in maize breeding and research. However, using this technology in major breeding programs with large numbers of haploids produced from a wide range of donors under different conditions, requires new inducers which combine: (i) a relatively high rate of haploid induction, (ii) a reliable system of marker genes allowing haploids to be identified, (iii) a good pollen production and seed set, and (iv) improved agronomic traits. Realization of these objectives was accomplished in four new haploid-inducing lines PHI (Procera Haploid Inducers), which were tested during the last three years. The maximal rate of haploid induction in one of the lines (PHI-1) was twice as high (15%) as in the best ancestral inducer - MHI (6-8%). Due to the combination of embryo (R1-nj) and root markers (Pl1 and B1) in the PHI inducers, haploids can be selected at three stages (dry kernels, 4-day old seedlings and mature plants) with a rather high accuracy. There was no negative correlation between haploid-induction ability and pollen production or seed set, which is a required for further improvement of inducers. The most important agronomic traits such as plant height, tassel size and lodging tolerance were significantly improved in PHI inducers. Results from haploid induction experiments of the PHI-3 inducer at Iowa State University (USA) in 2013 were quite comparable with those obtained in Romania.

P156

Analysis of maize (Zea mays L.) seedling roots with a new high-throughput tool to connect seedling roots to adult roots

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The maize root system is crucial for plant establishment as well as water and nutrient uptake. There is substantial genetic and phenotypic variation for root architecture, which gives opportunity for selection. Root traits, however, have not been used as selection criterion mainly due to the difficulty in measuring them, as well as their quantitative mode of inheritance. Seedling root traits offer an opportunity to study multiple individuals and to enable repeated measurements per year as compared to adult root phenotyping. Currently no strong relationships between seedling and adult root traits have been established with the traits and tools available so far. Our new tool is based on graphs, is fully automated, and is extendable to 3D tomography imagining data. It will enable capture of various traits from a single image of seedling roots. In order to evaluate this tool, a subset of the 384 inbred lines from the Ames panel, for which extensive genotype by sequencing data is available, was investigated. GWAS was applied to this panel for 27 traits captured from seedling root images using 135,311 SNP markers. This new software will enable capture of a large number of morphological root traits simultaneously, which may help to establish relationships between developmental stages between seedling and adult traits in the future.

Funding acknowledgement: Plant Sciences Institute, Iowa State University

P157

Analysis of the Causal Factors and Degree of Heterosis in the Haploid Induction Ratio in Maize

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The haploid induction ratio (HIR) in plants primarily depended by inducer, maternal genotype, and environmental conditions. The variation in HIR, the degree of heterosis, and the roles of inducers are still poorly understood in sweet corn (Zea mays L.) so far. We selected ten sweet corn maternal genotypes, and eleven inducers and six hybrids derived from these inducers, as female and pollen parents, respectively, in a field study in autumn 2010, spring 2011 and autumn 2011 in Guangzhou, China. The results showed that there was significant difference in HIR among inducers, which ranged from 0.41% to 4.20% with an average value of 2.47%. In contrast, the maternal genotype had no significant influence on mean HIR, which, in ten females, ranged from 2.02% to 6.28% with a mean value of 3.5%. In addition, the mean HIRs (MHIRs) of these ten female genotypes and of eleven inducers in autumn 2010 were 5.06% and 2.47% respectively. The corresponding MHIRs in spring 2011 were 1.73% and 2.09% respectively, which indicated that HIR was significantly influenced by environmental conditions. Among the six hybrids, only High Oil 1/Improved Stock6-1 displayed a positive value with respect to mid-parent heterosis (4.83%) but negative over-parent heterosis (-0.05%), while the other five hybrids displayed negative heterosis in HIR. In conclusion, haploid induction of sweet corn was more beneficial in autumn than in spring in Guangzhou of China. The inducers of HIR were significant in their effects, but the genotypes were not. Besides, the hybrids between inducers displayed higher and stronger stems, more pollen, longer inflorescences, and superior disease resistance than the parents. Thus we can conclude that hybrids between inducers can be widely used in haploid induction in sweet corn..

P158

Association Study of High Temperature Tolerance Traits in Maize

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Sporadic heat waves occurred in the past had caused significant reductions in food production worldwide. Maize plants are extremely sensitive to heat and drought, especially at stages critical for reproductive tissue development and yield production. The genetic variations for drought tolerance in maize and their underlying mechanisms have been studied extensively. However, little has been done about heat stress tolerance in maize. In this study, we characterized 26 NAM's founders for heat tolerant traits in the last few years and developed accurate phenotype rating systems for various heat tolerant traits that are easy to use for field-based phenotype evaluation. We also characterized the genetic variations for heat tolerant traits in a diversity panel containing a maize inbred line association panel and exPVPs (537 lines) in 2012 and 2013. The study has showed that maize germplasm possess a wide range of diversity of the responses to high temperature stress during both vegetative and reproductive developmental stages. A Genome-Wide Association Study (GWAS) of the resulting phenotype data is underway to identify chromosome regions and/or genetic loci associated with heat tolerance in maize germplasm. The findings of this study will provide information about beneficial alleles contributing to high temperature tolerance in maize; hence help to design better traditional and molecular breeding strategies for developing multi-stress tolerant hybrids.

Funding acknowledgement: United States Department of Agriculture (USDA)

P159

Characterization of the Sugarcane Mosaic Virus *Scmv2* Resistance region by Positional Association analysis in Maize

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Sugarcane mosaic virus (SCMV) is one of the most severe viruses in maize worldwide, resulting in serious lost in grain and forage yield in susceptible cultivars. In this study, two association panels consisting of 94 inbred lines each from China and US were characterized for resistance to two isolates: SCMV-Seehausen and SCMV-BJ. The population structures of both association panels were analyzed by using an Illumina Golden Gate Assay with 3072 single nucleotide polymorphisms (SNPs). 84 lines of Chinese panel were assigned to two sub-populations. The U.S. panel was subdivided into two sub-populations corresponding to Stiff Stalk Synthetic lines and Non Stiff Stalk Synthetic and tropical lines. The relative kinships were calculated using 2947 informative SNPs with minor allele frequencies ≥ 5% and missing data ≤ 20% for Chinese panel and 2841 for US panel, indicating that most lines had no or weak relationships with the others within panels. The *Scmv2* region was genotyped by using 12 single sequence repeat (SSR) markers by capillary electrophoresis. For all traits, the MLM model controlling both population structure and relative kinship ($Q + K$) was used for association analysis. Two SSRs DJF004 and 207FG003, located in the Scmv2 region were significantly associated with SCMV resistance, and explained 21.7% and 18.5% of phenotypic variation. Functional validation of promising candidate genes is ongoing.

Funding acknowledgement: RF Baker Center for Plant Breeding, China Scholarship Council

P160

Characterize the genetic basis of maize leaf traits using a large maize-teosinte population

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Plant architecture is crucial for grain yields by affecting light capture for photosynthesis and nitrogen storage for grain filling. Leaf traits including leaf size, leaf number and leaf orientation are main components of plant architecture. Although a large number of maize mutants in leaf development have been cloned and characterized, recent study demonstrated that the natural variation of maize leaf traits are dominated by large numbers of small-effect QTLs and the molecular mechanism underlying them remains largely unknown. During the domestication from teosinte to maize, in addition to the dramatic morphologic changes, leaf also experienced significant change. In this study, we used a large maize-teosinte BC2S4 recombinant inbred line (RIL) population that has been well constructed previously to investigate the genetic basis of three leaf traits (leaf length, leaf width and the length of leaf sheath). The goals of this study are to (1) Identify QTLs for leaf traits in maize-teosinte population and compare the results to the previously published mapping results using nested association mapping (NAM) populations; (2) Fine map and finally clone new QTLs to uncover the underlying molecular mechanism; (3) Evaluate if the loci/genes of interest are under adaptive selection. In summary, a total of 29 QTLs were detected for three traits and they can explain about 40% phenotypic variation of each target trait. Eighteen of the mapped QTLs are overlapped with the support interval of those reported in NAM population, showing remarkable consistency. As demonstrated previously, three maize leaf traits showed very little genetic sharing, suggesting that they are controlled by different set of genetic loci. A major QTL on chromosome 5 for leaf length (*qLL5*) that can explain 14% phenotypic variation was selected for further fine mapping. The preliminary fine mapping results will be presented. This study will further enhance our understanding of the genetic architecture of natural variation of maize leaf traits and set the basis for the characterization of important loci.

Funding acknowledgement: National Natural Science Foundation of China (NSFC), National High-tech Research and Development Projects (863)

P161

Cloning and Characterization of a QTL for salinity tolerance in maize

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Salt stress is one of the major constrains limiting crop productivity. High soil salt condition restricts water uptake and causes disorder in ion homeostasis. Molecular breeding provides an effective means to improve salinity tolerance in major crops. Through Genome-Wide Association Studies (GWAS) and QTL mapping with bi-parental populations, a major QTL for “early growth vigor” was identified from a collection of Pioneer germplasm. Soil testing and hydroponic culture under high NaCl concentration determined that the observed poor “early growth vigor” phenotype was caused by susceptibility to high soil salt concentration, and the QTL identified confers salinity tolerance in maize. Taking the map-based cloning approach, a candidate gene for the QTL has been identified. A deletion in the coding sequence in the susceptible allele of the candidate gene is the likely causative mutation. Functional complementation and allelism test are underway to validate the candidate gene. Gene expression analysis, alternative splicing patterns and other molecular characterization of the candidate gene will be presented.

Funding acknowledgement: DuPont Pioneer

P162

Combining large recombinant inbred lines and ultra-high density linkage map to dissect the plant architecture traits in maize

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The population size and density of marker are two important factors that limit the resolution of quantitative trait locus (QTL) mapping. In this study, we constructed and phenotyped a recombinant inbred lines (RILs) population with more than 1000 lines, and constructed a high density bin map by genotyping by sequencing (GBS) based single-nucleotide polymorphisms (SNPs). The map quality was verified by mapped four maize anthocyanin biosynthesis related genes *P1, pl1, bz1, r1* for silk color. Via QTL analysis for five plant architecture traits, we got 78 QTLs distributed across 10 chromosomes, including *ra1* for tassel branch number, *na1* for relative plant height, *br1, brd1* for ear height. Many of these QTLs were exactly defined to relative small genomic regions. Segments transmission analysis indicated that the plant height QTL located in the long arm of chromosome 5 is probably the major dwarf gene source in Chinese Zheng58 related germplasm. These results demonstrate the effectiveness of high resolution QTL mapping by combining large RILs and ultra-high density linkage map, and may helpful for further fine mapping, thus beneficial to molecular breeding.

P163

Discovery of a candidate gene underlying the QTL-*qMrdd1* that confers recessive resistance to maize rough dwarf disease

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Maize rough dwarf disease (MRDD) is a devastating viral disease, resulting in considerable yield losses worldwide. It is of vital importance to clone the resistance gene for development of MRDD resistant hybrids. A total of 50 F_9 heterogeneous inbred families (HIFs) were derived from two F_5 sibling plants (derived from a hybrid CL1165) contrasting at MRDD resistance. The 50 HIFs were genotyped using the MaizeSNP50 BeadChip (56,110 SNPs) and evaluated for their resistance to MRDD in the field. The trait-marker association study implied six chromosomal regions that were putatively associated with MRDD resistance. A major resistance QTL, *qMrdd1*, was detected and mapped on chromosome 8 in two segregating populations developed from four HIFs. By applying recombinant-derived progeny testing to self-pollinated backcrossed families, we fine-mapped the *qMrdd1* locus into a 208-kb region. Meanwhile, a collection of 335 F_6 recombinant inbred lines (RILs), derived from a Chinese hybrid Nongda108, was also used to map the*qMrdd1* locus and this mapping effort restricted the *qMrdd1* locus into a 385-kb region, covering the above 208-kb *qMrdd1* interval. The *qMrdd1* locus acted in a recessive manner to reduce the disease-severity index (DSI) by 24.2-39.3%. Thereafter, we screened the resistant 1145 BAC library and sequenced the positive BAC clones. Sequence alignment between the resistant 1145 and susceptible B73 within the *qMrdd1* region revealed a single candidate gene. Interestingly, the functional allele was detected in the susceptible B73 inbred line, while the null allele resulting from a TE insertion in the coding sequence was present in the resistant 1145 line. In addition, the *qMrdd1* locus did not show any negative linkage drag on morphological traits. A co-dominant marker was developed to diagnose the resistance *qMrdd1* allele, which has been used to assist introgression of *qMrdd1* into ten elite inbred lines to enhance maize resistance to MRDD.

Funding acknowledgement: National High-tech and development Program of China

P164

Dissect the genetic basis of maize inflorescence domestication

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The maize inflorescence (tassel and ear) experienced profound phenotypic changes during the domestication from its wild progenitor teosinte（*Zea mays* ssp. *parviglumis*）. Although a few key genes controlling such dramatic morphologic changes have been cloned, the genetic regulatory mechanism remains largely unknown. In this study, a large maize-teosinte BC_2S_4 recombinant inbred lines (RILs) population that has been well characterized previously was used to dissect the genetic architecture of maize tassel and ear. Seven tassel traits (primary branch number, secondary branch number, tassel length, main spike length, lateral branch length, length of branch zone and tassel branch angle) and seven ear traits (kernel row number, 100-kernel weight, cob weight, ear weight, cob length, cob diameter and ear diameter) were investigated. Combining with high-density SNP markers, QTL mapping for these 14 tassel and ear traits were conducted. The results showed that the genetic architecture of these tassel and ear traits varied greatly. Five tassel traits and six ear traits are controlled by a large-effect QTL plus many small-effect QTLs, whereas the remaining three traits are controlled by many small-effect QTLs. Interestingly, several hotspots controlling multiple inflorescence traits were identified. For example, the hotspot on chromosome 7 is specifically associated with multiple tassel traits, suggesting its important role during tassel domestication. *RA1*, a gene that has been cloned to be responsible for branching in tassel and ear, is the best candidate for this hotspot. Another hotspot on chromosome 5 appeared to be specifically associated with multiple ear traits, suggesting this locus may have played important role in ear domestication. A region on chromosome 10 was found to be associated with both tassel and ear traits. A recently cloned gene, *ZmCCT*, which is found to play key role during maize adapting to temperate environment, is located in this region and its pleiotropic effect is probably the reason for multiple traits association. The further characterization of hotspots and QTLs for tassel and ear traits will enhance our understanding of maize inflorescence domestication.

Funding acknowledgement: National Natural Science Foundation of China(NSFC), National High-tech Research and Development Projects (863)

P165

Dissection the genetic basis of maize kernel weight and shape

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Maize is one of the most important staple foods in the world. Kernel weight and shape are important components of grain yield and also key target traits during the domestication and breeding process. Here, we used 10 recombinant inbred lines (RIL) populations to dissect the genetic basis of maize kernel weight and shape with high-density genetic markers. All materials were planted in 4 locations over 2 years. BLUP values of each line were used to performed linkage and joint-linkage mapping. We identified 49, 39, 38, 46 and 42 QTLs through linkage mapping in single population for hundred-kernel weight, kernel test weight, kernel length, kernel width and kernel thickness, respectively, consisting of 82 unique QTLs. Of these unique QTLs, 24 can be regarded as major QTLs which can explain more than 10% of the variation of the traits investigated. We also conducted joint-linkage mapping and detected 39, 31, 32, 31 and 31 significant loci for hundred-kernel weight, kernel test weight, kernel length, kernel width and kernel thickness. As for hundred-kernel weight, these 39 significant loci can explain 43.7% of the total phenotypic variation. We are fine mapping and cloning a number of major QTLs that will help to explore the genetic basis and domestication process of maize kernel weight and shape.

Funding acknowledgement: This research was supported by the National Hi-Tech Research and Development Program of China and the National Natural Science Foundation of China

P166

Emergence under chilling stress of maize inbred lines by Genome Wide Association Study (GWAS)

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Maize originated in the tropical and subtropical regions, belongs to the thermophilic crops. Chilling stress has become the mainly limited factors of corn production. In this study, around six hundred inbred lines included Ex-PVP inbred lines, public US inbred lines and elite inbred lines of from China were used for GWAS and chilling tolerance-related traits evaluations. We evaluated chilling-tolerance responses(emergence percentage), 100-seed weight, average time to emergence, seedling fresh weight, seedling dry weight, shoot length, and single plant fresh weight of 600 maize inbred lines seeds treated in a cold room maintaining at 10±0.5 ℃ for 15 days and then moved to green house.

Totally, ten Million high quality SNPs were identified by re-sequencing 600 inbred lines and were selected for GWAS. We observed that the inbred lines percentage emergence, presents normal distribution in the natural population. The inbreds whose emergence percentage less than 10% originated from P group, while the emergence rate more than 90% belong to Lancaster, Tangsipingtou and Reid groups. Candidate genes associated with seed germination were found on chromosome1, 4, 8, respectively. We found that the genes contained B3 domain structure, ubiquitin ligases catalytic site and PPR domain structure which were related to seed germination and abiotic stress.

Funding acknowledgement: 973 program of China

P167

Evaluation of inducing parthenogenesis ability for different inducers in maize

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Maize doubled haploid breeding as one of important breeding technologies in maize, can make homozygous lines rapidly, shorten the period of breeding and improve breeding efficiency, it has been widely applied in maize breeding in the world. It is a precondition of this technology to select the inducers with high induce rate. This experiment has carried out at Hainan in 2012, fifty inducers from ten different basic populations were selected as males and Zhengdan 958 hybrid was choosed as female, three crosses has been made for each inducers. The result showed that the significant difference existed different inducers, the variation coefficient of induce rate for fifty inducers is 47.28%, the induce rate of inducers 283-2、287-6、283-4、287-4、287-2、279-11、287-9 and 287-3 are above 10%, especially inducer 283-2 with highest induce rate of 14.12%, while that of inducers 282-13、286-10、282-1、 286-3 and 286-6 is below 3%, especially inducer 286-6 with lowest induce rate, only for 1.16%. The induce rate of ten basic populations showed highly difference, the induce rate of all inducers from No.9 are above 10%, the average is 11.26% , the following is from No.2 with average induce rate of 9.74%, while the lowest induce rate of inducers is from No.8, only for 2.52%. The induce rate among 3 crosses for the same inducer showed significant difference, the variation coefficient of induce rate for inducers 283-4、279-8and280-1are below 5%, while for inducers 277-5、286-6、286-3 and 288-2 above 50%, which indicated the induce rate was affected enviormental factors except for genotypes.

Funding acknowledgement: National Science Foundation (NSF)

P168

Expression analysis of chlorophyll-degrading related genes during dark-induced leaf senescence in maize

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Chlorophyll, the most abundant pigment on earth, plays an important role to harvest luminous energy and drive electron transform, and its degradation is the most obviously characterize of leaf senescence, but it will generate dangerous molecule as reactive oxygen species. So understanding of the chlorophyll degradation became the focus of chlorophyll research. In the last two decades, many articles about chlorophyll degradation in higher plants had been published. In the model plant-Arabidopsis, all chlorophyll-degrading related genes had been cloned and characterized. But in maize, there were just one gene PAO (also named Lls1) have been reported, there was much work to be done. In this study, we obtained five homology genes involved the chlorophyll degradation pathway in maize by comparison of protein sequence from Arabidopsis. Our aim is to discover the expression regular pattern of chlorophyll-degrading related genes during leaf senescence through expression analysis of these genes during dark-induced leaf senescence in maize inbreds from NSS, SS and TST populations.

Funding acknowledgement: National Science Foundation (NSF)

P169

Fine mapping of *qAC6* and epistasis analyzing with *qAC10* controlling anthocyanin biosynthesis of kernel in maize

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Anthocyanins are water-soluble pigments which constitute the color of the plant petals, leaves, fruits, seeds and other organs. Numerous studies indicated the potential effects of anthocyanins may decrease the incidence of cardiovascular disease, cancer, hyperlipidemias and other chronic diseases if people eat high anthocyanin content foods. As a most important cereal crop in the world, maize with high anthocyanin content plays a significant role in human health. In this paper, the inbred line SDM with high anthocyanin content and the inbred line Mo17 with low anthocyanin content were used as parents to construct near-isogenic lines (NILs) for mapping *qAC6* and BC_4F_3 segregation population for analyzing QTL interaction by using foreground and background selection. One QTL-*anthocyanin content6* (*qAC6*) located on chromosome 6 were fine mapped at an interval around 114kb flanked by umc1014-S73. The analysis on epistatic effects between *qAC6* and the other QTL-*qAC10* which also controlled anthocyanin biosynthesis of kernel showed that the additive effect of *qAC6* was lower than that of *qAC10*, whereas the dominant effect of *qAC6* was higher than that of *qAC10*. The epistatic effect between the two QTLs was more important than additive effect of *qAC6* or *qAC10*. The plants with *qAC6* and *qAC10* produced much higher anthocyanin content kernels than those with *qAC6* or *qAC10*alone. These results provided scientific basis for cloning and characterizing *qAC6* and for breeding maize variety with high anthocyanin content.

Funding acknowledgement: Major Research Projects of Chongqing(cstc2012ggC80003, cstc 2012ggC 80004, cstc2012ggC 80006) , Fundamental Research Funds for the Central Universities (XDJK2013C023)

P170

Fine-mapping and Cloning a Major Effect Kernerl Row Number QTL *qKRN4e* in Maize

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Kernel row number (KRN) per ear was an important yield component, which directly affected grain yield by kernel number per ear in maize. During demonstration and improvement, KRN was dramatically altered, from 2 rows in teosinte to 8-20 rows in modern maize. We conducted a genome wide association analysis KRN employing global diversity inbred lines using high density SNP markers identified nine SNPs significant associated with KRN and formed a linkage disequilibrium (LD) block on chromosome 4 named as *qKRN4e*. Major effect QTLs were also detected by three linkage mapping populations. We used a large BC4F2 population (~10000) drived H21 X NX531 to screen recombination individuals in *qKRN4e* region. Base on the phenotype of recombination individuals, we fine-mapped *qKRN4e* to a ~4kb interval downstream an SBP-box genes. A mutant with a Mu7 insertion in promoter region of the SBP-box gene was acquired from UniformMu, which showed significant change in tassel branch and slightly change in KRN. Further, this SBP-box gene was highly expressed in female inflorescence when paired spikelet meristems (SPMs) and spikelet meristems(SMs) differentiated, implicating which might regulate KRN by modulating SPMs and SMs formation in immature ears.

Funding acknowledgement: National Basic Research Program of China (2009CB118400), the National Hi-Tech Program of China (2012AA10A307) and the National Natural Science Foundation of China (31271738)

P171

Generation mean analysis for grain yield among three maize heterotic groups

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Generation means analysis (GMA) is a method widely used to detect gene actions for complex or quantitative traits in plant breeding purposes. The information of additive, dominance, and epistasis genetic components for a trait is useful for maize breeders to design breeding programs for inbred line and hybrid development. Correlation between these genetic parameters and heterosis could further help breeders to select which inbred lines to be used for developing hybrids according to which genetic component is more important for a target trait from generation mean analysis. Six maize inbred lines from three heterotic groups had been used to make 5 sets of family with six generations of P1,P2,F1,F2, BC1,and BC2. Linear regression model was used to estimate additive, dominance, and epistasis genetic effects with Haymen (1958) genetic model for grain yield (GY) and five yield components of ear diameter (ED), ear length (EL), row of ear (RE), Kernel per row (KR), 100-kernel weight (KW) . Objectives of this study were to investigate 1)if genetic components of additive, dominance, and epistasis are different among different families for GY and five yield components (YCs); 2)which genetic components are more important for different traits;3) if heterosis is correlated to any genetic components and different relationships exist for different traits studied. Results showed that different genetic components contributed differently for GY, ED, EL, RE, KR and KW. The heterosis values were correlated mainly to dominant and epistasis components. Line selection strategies were discussed for super hybrid development by selecting appropriate crosses among different heterotic groups.

Funding acknowledgement: National Science Foundation (NSF)

P172

Genetic and agronomic assessment of cob traits in corn under low and normal nitrogen management conditions

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With rising energy demands and costs for fossil fuels, nitrogen prices are likely to increase and alternative energy from renewable sources such as maize cobs will become competitive. Maize cobs have beneficial characteristics for utilization as feedstock including compact tissue, high cellulose content and low ash and nitrogen content. In this study,quantitative trait loci (QTL)have been analyzed for cob morphological traits such as cob weight (WEI), cob volume (VOL),cob length(LEN), cob diameter (DIA) and cob tissue density(DEN), and grain yield(GY) under normal and low nitrogen regimes. 213 doubled haploid lines of the intermated B73xMo17 (IBM) Syn10 population have been resequenced for 8575 bins based on SNP markers. A total of 138 QTL were found for six traits across six trials using composite interval mapping with ten cofactors and empirical comparison-wise thresholds (P=0.001). Despite moderate to high repeatabilities across trials, few QTL were consistent across trials and overall levels of explained phenotypic variancewere lower than expected some of the cob trait x trial combinations (R^2 = 7.3–43.1%). Variation for cob traits was less affected by nitrogen conditions than byGY.Thus,the economics of cob usage under low nitrogen regimes is promising.

Funding: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P173

Genetic Architecture of Vascular Bundle In Stem During Maize Domestication

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Plant vascular bundle, composed of xylem, phloem and cambium, is evolved into transporting water, solute minerals, photosynthetic products and various signaling molecules and thus plays an essential role in plant growth and development. Great progresses have been made in the genetic basis of source and sink traits, however, the flow traits that link the source and sink and ensure the realization of yield potential remain largely unknown. The vascular bundle is the major component of flow. In this study, we used a large maize-teosinte BC_2 S_4 recombinant inbred line (RIL) population that has been well constructed previously to dissect the genetic architecture of vascular bundle number in stem. To score the phenotype, the uppermost internodes of mature stem for each RIL were collected, sliced, stained and scanned to count the number of vascular bundle. Combining the high-density SNPs, QTL mapping for vascular bundle number was conducted by R/QTL. A total of 17 QTLs were detected and they could jointly account for about 52.3% of the total phenotypic variation. Each individual QTL can only explain 1.7%-5.6% phenotypic variation, suggesting the vascular number in stem is dominated by large number of small-effect QTLs. Interestingly, 14 out of 17 QTLs, the alleles from teosinte appeared to increase the vascular number, suggesting that vascular number was probably under strong directional selection during maize domestication. The QTL with biggest effect located on chromosome 9 (*qVb9-1*) was chosen for further fine mapping. The teosinte allele at *qVb9-1* can increase about 20 vascular bundles. Through constructing isogenic lines, *qVb9-1* was preliminarily narrowed down to a 4Mb physical region. This study provides a starting point to understand the genetic basis of maize flow and the molecular mechanism underlying the flow changes during maize domestication.

Funding acknowledgement: Funding acknowledgement: National Natural Science Foundation of China (NSFC), National High-tech Research and Development Projects (863)

P174

Genetic association of nitrogen use efficiency and root-related traits in a RIL population of maize

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Nitrogen (N) is one of the most quantitatively important nutrients limiting crop growth and yield. However, less than 30% of N applied was utilized by crop plants, resulting in an inefficient use of resources and serious damage of the environments. Development of maize varieties with higher N use efficiency (NUE) will be a promising approach to ensure a sustainable maize production system. Although several studies highlighted the essential role of maize root system in N acquisitions, the genetic association between NUE and root-related traits remain to be elucidated. Here, we generated a maize RIL population derived from two parents that differed in both root architecture and NUE. Using this population, we evaluated NUE- related traits in the field and root-related traits in hydroponics under two contrasted N levels. Phenotypic and genotypic correlation between NUE and root traits, and the corresponding QTLs were determined. The results showed that root traits had a significant correlation with those of NUE and NupE, and several QTLs for roots were co-localized with NUE and NupE, in particular a QTL cluster on chromosome 3 (3.04). Together, the results revealed genetic relationship between root-related traits and NupE, and highlighted the most promising genetic region for molecular breeding for N efficient maize varieties.

Funding acknowledgement: 863 program（2012AA100304); National Natural Science Foundation of China(31172015)

P175

Genetic control of leaf width variation by *Cellulose Synthase-Like D1*

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Leaf width is an important component of leaf architecture, which directly determines the light absorption, and in turn affects grain yield in maize. Here, a F_2 population was developed from a cross between an inbred line with narrow leaf, mt03-1, and an inbred line with wide leaf, LEE-12, and genotyped using 3072 SNPs. An extremely large QTL for leaf width (LOD = 83.6, R^2 = 80%) was identified in the genomic region from 8.93 to 34.9 Mb on chromosome 10. Using about 4,000 BC_2F_1 plants, the QTL interval was narrowed down to 3.29-Mb region, within which fell the *Cellulose Synthase-Like D1* (*CslD1*) gene regulating the width of organs in previous studies. Sequencing the entire gene showed that a 467-bp insertion and a 63-bp deletion in the third exon resulted in the loss of the function of *CslD1* by frame shift. Although the cell-level effects of the *csld1* mutant on organ width was elucidated, the regulation of the leaf width variation by *CslD1* remains unclear.

Funding acknowledgement: National Natural Science Foundation of China（NSFC）

P176

Genetic dissection and fine mapping of rice QTLs for panicle length and evaluation of their potential to increase yield

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As the major cereal of Asia, rice provides staple food for nearly half of the world's population, and the yield improvement becomes the primary target in rice breeding. Panicle morphology is key element in determining yield, including primary branch number, secondary branch number, panicle density and panicle length. Crescent studies have unraveled the genetic basic about the first three components but little knowledge about panicle length is obtained. To find out the loci for panicle length, we performed QTL analysis in several backcrossed populations which showed segregated phenotype. Two QTL loci were identified on chromosome 6 and 8 separately, and named qPL6 and qPL8. Alleles for increasing panicle length come from respective original varieties, Nipponbare and WS3, a landrace stock bearing large panicle and strong culms, that is, qPL8 from Nipponbare and qPL6 from WS3 contribute to the increased panicle length. High-resolution mapping was carried out to facilitate the chromosomal location of qPL6 and qPL8. Finally, utilizing several key recombinants, qPL6 was constrained to about 25Kb by adjacent makers and qPL8 to about 375Kb, laying the foundation for gene identification and functional study. To clarify the relationship between panicle length and other yield components, NILs of qPL6 and qPL8 were developed from the same backcrossed population. Compared with lines harbouring opposite allele, no significant difference was found for plant height and heading date but two loci showed distinct characteristics for panicle morphology. In addition to panicle length, the qPL6 locus could form more and longer primary branches and then more secondary branches, which made one panicle produce more spikelets and had the potential to increase yield. However, no pliotropic effect was found for qPL8, and when pyramided with qPL6, the effect was additive. This work will provide genetic resources for modulating rice panicle in variety development.

P177

Genetic dissection of tocopherol concentration in maize kernel combining linkage and association analyses

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Known as vitamin E, tocopherols play an important role both in protecting the photosynthetic system in plants and as an antioxidant in human body. In maize kernel, tocopherols mainly exist as γ-tocopherol, α-tocopherol and δ-tocopherol. Six populations of recombinant inbred lines (RILs) were utilized to conduct linkage analysis using high-density linkage maps generated from Maize SNP50 array. An association mapping panel of 513 inbred lines was used for genome-wide association (GWA) analysis. The three forms of tocopherols were extracted from the maize kernel and measured by ultra-performance liquid chromatography (UPLC). In total, 39 unique quantitative trait loci (QTL) were identified, explaining 2.9%-48.7% of variation on tocopherol concentration or composition. Among them, 12 were regarded as major QTLs, each explaining greater than 10% of phenotypic variation. Six major QTLs were identified in multiple populations including the previously cloned *ZmVTE4*, while the other six were only found in single populations, suggesting rare allelic variations may underlie the QTLs. In association analysis, 25 significant sites fell in the QTL intervals. Combined with transcriptomic data derived from RNA sequencing as well as functional annotation of candidate genes, several candidate genes were selected for further analysis. Fine mapping of major QTLs are being carried out taking advantage of heterogeneous inbred families (HIFs) obtained from the RIL populations. Our results implies that tocopherol concentration in maize kernel is decided by a few major QTLs and a number of modest ones. For rare variations, association analysis only has limited power. On this condition, combination with linkage analyisis to clone a number of major loci will assist us to unveil the genetic architecture of tocopherol variations.

Funding acknowledgement: National High Technology Research and Development Program of China (863 Program), Huazhong Agricultural University Scientific & Technological Self-innovation Foundation

P178

Genome Wide Association Studies using a new nonparametric model reveal the genetic architecture of 17 agronomic traits in an enlarged maize association panel

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Association mapping is a powerful approach to dissect the genetic architecture of complex quantitative traits using high-density SNP markers in maize. Here, we expanded our association panel size from 368 to 513 inbred lines with 560K high quality SNPs using a two-step data-imputation method which combines identity by descent (IBD) based projection and k-nearest neighbor (KNN) algorithm. Genome-wide association studies (GWAS) were carried out for 17 agronomic traits with a panel of 513 inbred lines applying both mixed linear model (MLM) and a new method, the Anderson-Darling (A-D) test. Only 10 loci and none affecting five among 17 measured traits were identified using the MLM method at the Bonferroni-corrected threshold -log P>5.74=1/n and -log P>7.05=0.05/n respectively. Many more loci ranging between 1 and 35 loci (107 loci for plant height) were identified using the A-D test at the Bonferroni-corrected threshold (-log P>7.05=0.05/n). Many known loci and new candidate loci were only observed by the A-D test, a few of them were also detected in independent linkage analysis. This study indicates that combining IBD based projection and KNN algorithm is an efficient imputation method for inferring large missing genotype segments. In addition we show that the A-D test is a more powerful tool for GWAS than existing methods, and provides a rich resource for maize genetics and breeding.

Funding: National Natural Science Foundation of China ; National Hi-Tech Research and Development Program of China

P179

Genome wide genetic marker discovery and genotyping using next-generation sequencing in maize IBM Syn10 mapping population

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In order to safeguard feeding the growing human population, it is important to understand and exploit the genetic basis of quantitative traits. Next-generation sequencing technology had shown advanced advantage and effective performance in genetic mapping and genome analysis of diverse genetic resources. In this study, with deep re-sequencing of Mo17 genome, millions of high quality SNPs between Mo17 and B73 were detected and further verified in maize Intermated B73 × Mo17 (IBM) Syn10 population to well complement the existing database. Moreover, 280 lines of IBM Syn10 population were re-sequenced with average 0.31x coverage to construct an ultra-high density genetic bin map based on the parental SNPs and sliding window approach. Combined with IBM Syn4 RIL population, we detected 135 QTLs for flowering time and plant height traits across two populations. Eighteen functional known genes and twenty-five candidate genes were fine mapped, showing a high precision in quantitative trait mapping. Map expansion and segregation distortion were also analyzed, and evidence for inadvertent selection of early flowering time in the process of mapping population development were observed. Our results indicate increased quality and accuracy of bin mapping for detecting QTLs and dissecting genetic variation in IBM Syn10 compared to Syn4 population. Furthermore, an updated integrating map with 1,151,856 high quality SNPs, 2,916 traditional markers and 6,618 bin markers were constructed. Thus, our findings provide a fundamentally genetic data for cost-effective QTL mapping in an updated IBM Syn10 population and provide a reliable and verified high quality SNP set between Mo17 and B73 for molecular breeding in future.

Funding: The work was supported by grants from the National Natural Science Foundation of China (31271740), the National Hi-Tech program of China (2012AA10307), and the Major Project of China on New varieties of GMO Cultivation (2011ZX08003-003)

P180

Genome-Wide Analysis of *ZmDREB* Genes and Their Association with Natural Variation in Drought Tolerance at Seedling Stage of *Zea mays* L.

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The worldwide production of maize (*Zea mays* L.) is frequently impacted by water scarcity and as a result, increased drought tolerance is a priority target in maize breeding programs. While DREB transcription factors have been demonstrated to play a central role in desiccation tolerance, whether or not natural sequence variations in these genes are associated with the phenotypic variability of this trait is largely unknown. In the present study, eighteen *ZmDREB* genes present in the maize B73 genome were cloned and systematically analyzed to determine their phylogenetic relationship, synteny with rice, maize and sorghum genomes; pattern of drought-responsive gene expression, and protein transactivation activity. Importantly, the association between the nucleic acid variation of each *ZmDREB* gene with drought tolerance was evaluated using a diverse population of maize consisting of 368 varieties from tropical and temperate regions. A significant association between the genetic variation of *ZmDREB2.7* and drought tolerance at seedling stage was identified. Further analysis found that the DNA polymorphisms in the promoter region of *ZmDREB2.7*, but not the protein coding region itself, was associated with different levels of drought tolerance among maize varieties, likely due to distinct patterns of gene expression in response to drought stress. In vitro, protein-DNA binding assay demonstrated that ZmDREB2.7 protein could specifically interact with the target DNA sequences. The transgenic Arabidopsis overexpressing *ZmDREB2.7* displayed enhanced tolerance to drought stress. Moreover, a favorable allele of *ZmDREB2.7*, identified in the drought-tolerant maize varieties, was effective in imparting plant tolerance to drought stress. Based upon these findings, we conclude that natural variation in the promoter of *ZmDREB2.7* contributes to maize drought tolerance, and that the gene and its favorable allele may be an important genetic resource for the genetic improvement of drought tolerance in maize.

Funding acknowledgement: The National Hi-Tech Research and Development Program of China (2012AA10A306-4), the National Basic Research Program of China (2012CB114302-4), the National Natural Science Foundation of China (31171163) and the Chinese Academy of Sciences (CAS) project

P181

Genome-wide association and meta-analysis on a comprehensive panel of 4,500 maize accessions from CIMMYT's breeder's core collection

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Flowering time, the time of the transition from vegetative to reproductive growth, is an important trait for local adaptation of plants and in maize has been an important target for selection since domestication. Furthermore, flowering time in maize is a complex trait controlled by many loci of modest effect. In terms of breeding, linkage drag associated with flowering time limits the transfer of other traits across genetic pools.

QTL mapping and Genome Wide Association Studies (GWAS) have been performed to study flowering time in different germplasm pools of maize, however the representation of maize landraces in these analyses remains limited. The International Center for Maize and Wheat Improvement (CIMMYT) houses the largest collection of maize accessions in the world, and it has recently started an initiative called Seeds of Discovery (SeeD). SeeD is primarily supported by the Mexican Government to facilitate exploration and use of the diversity contained within this collection. In this project, a comprehensive sample of ~4,500 accessions from CIMMYT's maize collection, the "breeder's core collection", was genotpyed and phenotyped. Here I present results of GWAS for days to anthesis (DA), days to silking (DS), and the anthesis-silking interval (ASI) in the SeeD panel.

Phenotyping was performed in 2011 and 2012 (3 season, 23 trials) following an augmented row-column design. Accessions were evaluated according to their adaptation zone, and Best Linear Unbiased Predictions (BLUPs) were used to estimate breeding values. Genotyping was performed through Genotyping by Sequencing (GBS), which yielded a total of ~1 million SNPs. Genotypic data was imputed using TASSEL and BEAGLE4. GWAS and meta-analysis were performed for DA, DS, and ASI on the SeeD panel. Prior results for these traits on other populations were used to evaluate accuracy and statistical power. Maize landraces contain significant genetic diversity, and here we highlight the challenges and potential for using this material in quantitative genetics and plant breeding.

Funding acknowledgement: Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA), National Science Foundation (NSF) , United States Department of Agriculture (USDA)

P182

Genome-Wide Association Study and Linkage Mapping dissect the Genetic Architectures of Amino Acids in Maize Kernel

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Several hundred million people in the world rely on maize as their principal daily food and for feeding livestock and animals. However, it usually lacks of the full range of amino acids as lysine and tryptophan in normal cultivars. It is an essential task to detect the genes controlling amino acids content in maize kernel that will enhance high quality maize breeding. In this study, 513 diverse maize inbred lines and three recombination inbred line (RIL) populations were used to detect QTLs affecting each amino acid. Totally, 82 associated SNPs were identified ($p < 1.83 \times 10^{-6}$), dispersing in 30 genes across the whole genome. Among these genes, GRMZM2G015534 (*Opaque2*), had been confirmed to affect lysine content in maize kernel. The correlation between the SNP polymorphisms identified in this gene and mRNA expression level was significant ($p=3.01 \times 10^{-10}$, n=368), and the expression level was also significantly corrected with lysine content variation ($r=-0.27$, $p=4.72 \times 10^{-4}$). Moreover, 379 ($p<1.0 \times 10^{-4}$) among the 28769 genes with RNA-seq data were regulated by *Opaque2* gene. GO (Gene Ontology) enrichment analysis shown that there were two GO terms. One was zein related genes, the other was about plasma membrane. Totally, 17, 42 and 36 QTLs were identified in three RIL populations, respectively, only three QTLs of which were commonly detected in at least two RIL populations. Two loci identified by GWAS located in the confidence intervals of QTLs in this study, which were significant associated with lysine and arginine levels, respectively, and encoding deoxyuridine 5-triphosphate nucleotidohydrolase and signal peptide peptidase, respectively. More work is ongoing to identify the major QTLs and genes to dissect genetic basis of amino acids biosynthesis.

Funding acknowledgement: The National Hi-Tech Research and Development Program of China and the National Natural Science Foundation of China

P183

Genome-wide dissection of maize ear traits by multiple-parents population based linkage and association analyses

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Maize (*Zea mays*) is one of the most important staple crops in the world. Maize yield is highly correlated with the performance of ear and genetic dissection of ear traits will provide us informative clues to accelerate breeding of high yielding varieties. We developed ten RIL populations with 14 parental materials from 500 genetically diverse maize inbred lines. This germplasm were genotyped with 56,110 SNPs by MaizeSNP50 BeadChip while the 500 diverse lines were also genotyped with approximate 1.1 M high-quality SNPs by RNA sequencing and imputation. Through saturated genetic maps, the ultra-density genotypes of 14 parental lines were projected onto the RIL offsprings. All the materials were planted in eight environments in two consecutive years and three yield related traits including ear length, ear row number and ear weight were investigated.

We combined linkage and association analyses to dissect maize ear traits via different statistical models: 1) using composite interval mapping based linkage mapping, we detected 64, 68 and 43 QTLs for three traits, respectively, and most of QTLs were uniquely detected in specific genetic background; 2) using mixed model based joint linkage mapping, we identified 40, 54 and 67 QTLs for three traits, respectively; 3) using multiple regression based GWAS, 187, 153 and 106 SNPs were observed to be significantly associated with three traits, respectively. Majority of candidate SNPs showed a minor level of additive effect, but cumulatively explaining 61~84% of total phenotypic variation.

Based on the results of this study, we can conclude that maize ear traits may be attributed to numerous common and rare variants. The genetic design of joint populations enables us to enhance the power for common variants but not for rare variants. The high predictability of additive model demonstrates the great potential of applying these associated SNPs in molecular breeding program.

Funding acknowledgement: National Natural Science Foundation of China (NSFC), National High-tech R&D Program of China (863 Program)

P184

Grain moisture measurement based on Low-Field Nuclear Magnetic Resonance in maize (*Zea mays L.*)

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Maize (*Zea mays L.*) hybrids with fast dry down are desirable in corn production. Field drying rate was evaluated based on grain moisture variation during one period. To investigate the grain moisture of maize kernel, two mid-maturity hybrid (Zhengdan 958 and Pioneer 335) and one early maturity hybrid (Demeiya 2) were tested based on Low-Field Nuclear Magnetic Resonance (NMR) spectroscopy. We have developed a model which is suit for grain moisture evaluation ranges from 0% to 70% by NMR. The method was employed to survey grain moisture every 5 days from 30 days after pollination (DAP) to physiology maturity. Our results indicated: 1. The field drying rate of mid-maturity hybrid shows the tendency of fast-mid-fast. Grain moisture of Pioneer 335 dropped rapidly 55 DAP (2.13% decline per day, 55-60 DAP) compared with before (0.05% decline per day, 50-55 DAP). The feature means that maize kernels with fast dry down could be selected during this period. 2. Early maturity hybrid Demeiya 2 trends to be drying fast (1.42% decline per day, 35-40 DAP&1.47% decline per day, 40-45 DAP) across the grain filling period. 3. Grain moisture variation among kernels is widely existed, which means single kernel selection of fast dry down maize is feasible. After all, our work reveals that grain moisture could be measured accurately by NMR with proper model. Developing hybrid with high field drying rate through single kernel selection with this method could be possible.

Funding acknowledgement: National '863' High-Tech Program of China (Grant No: 2011AA10A103), Modern Maize Industry Technology System Foundation of China (Grant No: CARS-02-09)

P185

How corn got its spots: the genetics of lesions, disease and the defense response.

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Rp1-D21 is a maize auto-active resistance gene conferring a spontaneous hypersensitive defense response (HR) of variable severity depending on genetic background. Using the Mutant Assisted Gene Identification and Characterization (MAGIC) approach we have identified naturally-occurring allelic variants associated with phenotypic variation in HR. A set of genes predicted to play significant roles in the control of ubiquitin protein degradation, programmed cell death, autophagy and oxidative stress response pathways were identified. We are working to validate and characterize these genes and to delve deeper into the connections between defense response, lesion mimics and resistance to biotrophic and necrotrophic pathogens.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P186

Identification of Disease Resistance to Maize Rough Dwarf Virus in Core Inbred Lines

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Maize (*zea mays* L.) is one of the most important crops in terms of both grain and forage production across the world. It has become to the first one of the crops in China recent years. Maize Rough Dwarf Virus (MRDV) is a viral disease that results in substantial yield losses worldwide. The main viral pathogen of MRDV resourced from rice black-streaked dwarf virus (RBSDV) which is transmitted by planthopper insect. In post several years, MRDV occurred severely and became to critical factors that limited the maize yield in China. Most of the maize hybrids displayed susceptibility or weak resistance to MRDV. In order to genetically increase disease resistance to MRDV, 50 representative inbred lines which extensive used in maize breeding were evaluated for the resistance to MRDV. Of the 50 inbred lines，Q319 show a strong higher resistance to MRDV, Shen153, Shen137 and Dan598 with medium resistance，while 9801, 478, LY80, 65235 and XY420 displayed high sensitivity to MRDV. Other materials show different degrees of susceptibility. Preliminary genetics and molecular biology research demonstrated that the major resistant QTL of MRDV located in short arm of chromosome 10. However, the artificial inoculation of Maize Rough Dwarf Disease is unfeasible, which brings great challenges to the genetic studies on this resistance QTL. We are performing further researches on this QTL by combining molecular techniques and bioinformatics. It's expected to get the resistant gene cloning and find the co-segregation makers for molecular breeding.

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Intraspecific variation of recombination rate in Maize

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Meiotic crossovers ensure the proper segregation of chromosomes and shuffle intra-chromosomal allelic combinations. Such genetic reassortment is exploited in plant breeding to combine favourable alleles of genes. Using linkage or association-based approaches, meiotic crossovers are also exploited in genetic research to identify the genetic factors underlying the traits of interest. Crossover numbers and distributions along chromosomes vary from species to species but little is known about their intraspecies variation, though it would help optimizing breeding schemes by taking into account the global recombination rates and recombination landscapes when choosing the genotypes to be used as parents for crosses. Here, we report on the variation of recombination rates between 22 European maize inbred lines belonging to the Dent and Flint genetic groups. We produced 23 doubled-haploid populations derived from crosses between these lines and two central parents, UH007 (Flint) and F353 (Dent). All populations were genotyped with a 50k-SNP array and used to construct high-density genetic maps. Comparing each genetic map length to the physical length based on the B73 sequence, we found significant differences of genome-wide recombination rates among populations (0.57 to 0.74 cM/Mbp when pooling all chromosomes). Recombination rates also showed significant differences between chromosomes (0.60 to 0.88 cM/Mbp when pooling all populations). Using an additive genetic model, we estimated individual genetic values of recombination rate for the 22 parental lines, given the data obtained from the 23 double-haploid populations. Genetic values showed up to two-fold differences between parental lines, but no evidence for general differences between Dent and Flint material. Aligning each genetic map to the B73 sequence, intra-chromosomal recombination landscapes revealed regions with significant differences in local recombination rate between populations. Finally, crossover interference analysis using a two-pathway modelling framework revealed a negative association between recombination rate and interference strength.

Funding acknowledgement: ANR (France), BMBF (Germany), MINECO (Spain)

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Large-scale germplasm evaluation and candidate gene identification for low phosphorus tolerance in maize

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Low-phosphorus (LP) stress is a global problem that threatens food security. Germplasm evaluation and candidate gene identification for LP tolerance and their application in marker-assisted breeding would help farmers to cope with this problem. In this research, a total of 755 maize genotypes were used for screening LP-tolerant genotypes in the field condition in 2011 and 2012, which included 539 tropical and subtropical lines and 216 temperate lines. Significant genotypic variation was detected for various traits under both LP and normal-phosphorus (NP) conditions. Both tropical/subtropical and temperate ecotypes contained LP-sensitive and -tolerant lines, which can be used to improve LP tolerance. From the 83 re-sequenced inbred lines that were used in the field trails, nine tolerant and eight sensitive lines were identified. Comparing 26 million SNPs between tolerant and sensitive lines, we identified 16250 SNPs that were specific in at least eight tolerant lines or seven sensitive lines, with 121 SNP hotspots identified when the false discovery rate was set at 0.10. The hotspot regions contained 39 genes homologous to those previously identified to be involved in plant LP tolerance. A total of 1378 genes were found in the 5 kb surrounding regions at the 16250 SNP loci, and GO analysis revealed that the genes encoding protein kinase and sulfur-containing group transferase might be related to LP tolerance in maize. The gene identification strategy used in this study for quantitative traits provides an option for similar researches, and the genes identified would be useful for improving LP tolerance in maize after further characterization.

Funding acknowledgement: National High-Tech R & D Program, China Postdoctoral Science Foundation

P189

Mapping QTLs for Salt Tolerance Based on SNP Markers at Seedling Stage in Maize (*Zea Mays L.*)

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Salinity is a major constraint to the sustainability and expansion of maize cultivation. Salt tolerance is a quantitative trait controlled by multiple genes. In the present study, we constructed a high density genetic map based on high quality SNPs from 161 F2:5 RIL populations derived from the cross between two maize inbred lines contrasting in salinity tolerance. The parents and the RIL lines were grown in hydroponic system at seedling stage. After 7 days of 160 mM NaCl treatment, the salt tolerance rating (STR), fresh weight (FW), dry weight (DW), tissue water content (TWC), and the Na+ and K+ contents of the shoots (SNC, SKC) were measured. The QTLs for salt tolerance were determined by the IciMapping software, a total of 8 addictive QTLs and 18 epistatic QTLs were identified, including 3 additive QTLs and 3 epistatic QTLs for TWC, 4 additive QTLs and 5 epistatic QTLs for STR, 1 additive QTLs and 5 epistatic QTLs for FW, and 5 epistatic QTLs for DW. Of these QTLs, two major ones, qSTR-3 and qFW-3, explained 20.1% and 19.7% of the total phenotypic variance, respectively. The results are helpful for understanding the genetic basis of salt tolerance in maize and provide useful information for genetic improvement of salt tolerance in maize by marker-assisted selection.

Funding acknowledgement: National Science Foundation (NSF)

P190

Marker assisted selection of high oil in vivo haploid inducers in maize

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Double haploid (DH) technology can purify resources rapidly, which has gradually becoming one of the key technologies of modern maize breeding. In previous studies, a major quantitative trait locus (QTL) qhir1 was narrowed to 243kb, which make it feasible to use maker assisted selection (MAS) for inducers development. Recently, a new method on haploid identification by oil content (OC) was proved to be effective. So our objectives were to develop high oil inducers by MAS of qhir1 locus and evaluate the method of haploid identification by OC through newly developed high oil inducers. Population with F2, first BC to the inducer CAU5 (BC1F1-CAU5) and high oil inbred line GY923 (BC1F1-GY923) from the crosses GY923 × CAU5 were constructed and continuously selfing to develop high oil inducers. In each cycle three different parameters including kernel OC, marker genotype at qhir1 and haploid induction rate (HIR) were used for pedigree selection. Three lines were developed to be candidate high oil inducer lines with OC about 8.5%, HIR about 8% and superior agronomic performance, which were suitable for application of haploid identification by OC. We corroborated HIR selection joint with MAS for qhir1 was effective, which provide new insights for haploid inducer breeding. Besides, the accuracy for the haploid identification by OC was influenced by female germplasm resources and high oil inducers, and appropriate critical points for OC can balance the false discovery rate (FDR) and false negative rate (FNR).

Funding acknowledgement: Modern Maize Industry Technology System Foundation of China (Grant No: CARS-02-09), National '863' High-Tech Program of China (Grant No: 2011AA10A103)

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Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights

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We present a comprehensive study of maize metabolism, combining genetic, metabolite and expression profiling methodologies to dissect the genetic basis of metabolic diversity in maize kernels. A total of 983 metabolite features were quantified in 702 maize genotypes planted at multiple locations. Metabolite-based genome-wide association mapping (GWAS) identified a total of 1459 significant locus-trait associations ($p \leq 1.8 \times 10\text{-}6$) across three environments. Most (58.5%) of the identified loci were supported by expression QTL, and some (14.7%) were validated by linkage mapping. Potential causal variants of five candidate genes which were associated with five metabolic traits, respectively, were identified by re-sequencing and candidate gene association analysis. Function of two of the five genes was validated by mutant and transgenic analysis, respectively. All data are available as a public resource to aid functional studies and interpretation of GWAS findings. A number of the found metabolite features associated with kernel weight can be used as biomarkers to facilitate genetic improvement of maize.

Funding acknowledgement: National Hi-Tech Research and Development Program of China, National Program on Key Basic Research Project of China, National Natural Science Foundation of China

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Patterns of Genomic Variation in Chinese Maize Inbred Lines and Implications for Genetic Improvement

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Maize has an irreplaceable role in ensuring global food security, which is being challenged by increasing population and unprecedented consumption. The exploration of the genetic relationships, heterotic patterns, and breeding history of maize inbred lines plays a critical part in directing maize improvement projects. In this study, 273 elite temperate inbred lines, used in Chinese maize breeding programs, were genotyped using the Illumina MaizeSNP50 BeadChip containing 56,110 SNPs. The population structure and principle component analysis of these lines revealed the patterns of genomic variation in current Chinese breeding lines, which explain the historical role of five critical founder lines, Mo17, Dan340, Zi330, Ye478, and HZS, in the Chinese maize breeding program. Mo17 related commercial hybrids were firstly and widely used in 1970-1980, followed by the release of Ye478 and HZS in 1980-1990, and the introduction of "Tem/Trop I" or "P" heterotic groups in 1990-2000. Using these high-density SNPs, we also reconstructed the recombination events of elite lines, HZS and its six derived lines, and identified inbred line Dan340 as one important genome donor for Zheng58, which is the female parent of ZhengDan958, the most widely grown commercial hybrid currently in China. These results provide useful information for future maize breeding.

P193

Phenotype-selected introgression library for studying genetic architecture of plant height in maize

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Genetic architecture refers to the numbers and genome locations of genes that affect a trait, the magnitude of their effects, and the relative contributions of additive, dominance, and epistatic gene effects. Studies of genetic architecture are helpful for our understanding of plant evolution and the design of crop improvement strategies. Here, we established a collection of phenotypic introgression families to enrich for dominant acting genome segments and genes affecting plant height as quantitative genetic model trait in isogenic background as tool to understand the genetic architecture of a complex inherited model trait. Rather than representing one complete donor genome in a recurrent parent (traditional introgression method), our ambition is to enrich for chromosome segments affecting a single trait of interest (plant height). By utilizing phenotypic selection, we introgressed segments from multiple sub-tropical or tropical donors into two elite inbred lines, PHB47 and PHZ51, both used as recurrent parent in the germplasm enhancement in maize (GEM) projects. We established a collection of near-isogenic families enriched for genome regions affecting plant height (but not flowering time). SNP markers were employed to detect and utilize favorable alleles from exotic germplasm. We found that the observed donor genome proportion of the BC1 families in our library was significantly increased compared to an unselected BC1-derived panel of doubled haploid lines (Brenner et al., 2012, Mol. Breeding), and most families carrying donor chromosome segments were significantly ($P = 0.01$) taller than their respective recurrent parents, supporting efficiency of our selection method to establish a comprehensive basis for future efforts towards understanding the molecular basis of the quantitative trait plant height.

Funding acknowledgement: Agronomy department, Iowa State University

P194

Potentials of Arbuscular Mycorrhiza fungus Gigaspora gigantea in tolerating drought in maize (Zea mays L.)

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Four accessions of maize obtained from different market locations in O gun state of N igeria were used to investigate the influence of Arbuscular mycorrhizal fungi (Gigaspora gigantea) on the tolerance of maize to drought stress. The experiment was conducted in the teaching and research farm of the Department of Agriculture Babcock University, Ilishan-Remo, N igeria. The experiment was laid out in a complete randomized design with four replicates. Data were collected on eight morphological drought related characters. The combined analysis of variance showed significant ($P<0.05$) treatment effect on majority of the traits evaluated. The treatments of AMF produced significant higher growth related traits suggesting that AMF treated plants had high potential in influencing the tolerance to drought. Accession 3 was considered best for most of the traits studied and can be selected as parents in maize breeding for yield related components.

P195

QTL detection for grain water relations and genetic correlations with grain matter accumulation at four stages after pollination in maize

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Abstract: Grain water relations were closely correlated with matter accumulation during grain development. In this study, QTL mapping for grain water content (GWC) at four stages after pollination and grain dehydration rate (GDR) during six intervals were done using 258 recombinant inbred lines (RIL). Meta-QTL (mQTL) was revealed by meta-analysis using BioMercator for both traits herein and together with seven traits related with grain matter accumulation in our previous research. Among 40 QTL detected for GWC and 35 QTL for GDR, 45 QTL were stage/period specific. QTL on chromosome 5 could be considered as full-stage QTL. Eight of 11 mQTL included QTL for both traits. Grain matter traits were positively correlated with GWC, but negatively correlated with GDR in most cases. Low coincidences in QTL position and opposite allelic effects for two kinds of traits suggested that their simultaneous improvement might be realized. Selection for low grain moisture could be focused on QTL at bins 1.07-1.08, 2.08, 4.03-4.04 and 5.03-5.04, while it should be followed to QTL at bins 7.02-7.03, 1.03-1.04 and 10.05-10.06 for high grain weight. However, this should be proved through practical selection, and the related marker intervals needed to be narrowed down in further research.
Key words: Grain water relations; Grain matter accumulation traits; Genetic correlation; QTL detection; Meta-QTL analysis

Funding acknowledgement: Henan Innovation Project for University Prominent Research Talents (2005HANCET-12), Henan Natural Science Foundation (0511032900), Henan Development and Reform Commission

P196

QTL Mapping and Meta-analysis for Kernel Composition Traits across Three Generations in Popcorn

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Popcorn have become very popular snack across the world today as they are tasty, convenient to eat and also nutritious. It would be benefit to increase the edible quality of popcorn flake by improving major kernel composition traits. In this research, the RIL population with 258 recombinant inbred lines was evaluated to detect quantitative trait loci (QTL) for four kernel composition traits (CT, starch concentration; CP, protein concentration; CF, oil concentration; LS, lysine concentration) under three environments. Meta-analysis was used to integrate QTLs detected across three generations (RIL, F2:3 and BC2F2) derived from the same cross. A total of twenty-three QTLs were detected for four traits, on chromosomes 1, 2, 7, 8 and 10 for CF, on chromosomes 2, 3, 4, 5 and 6 for CP, on chromosomes 1, 3, 4, 5 and 9 for CT, and on chromosomes 1, 3, 5, 8 and 9 for LS. Four QTLs at bins 3.04, at bins 4.01 and at bins 5.03~5.04 were consistently identified in the similar chromosome locations in the RIL population. Three QTLs at bins 1.07~1.08, at bins 3.04 and at bins 6.07 were commonly identified in the same or near bins in all 3 generations. Fifteen meta-QTLs were identified on chromosomes 1, 2, 3, 4, 5, 6, 7 and 9. Most meta-QTLs included two or three traits, which reflected pleiotropic or tightly linkaged QTLs for the four kernel composition traits. Three chromosome locations, at bins 3.04, at bins 4.01 and at bins 5.03~5.04 with the higher contribution to kernel composition traits were consistently detected, which could be made further research to improve main compositions content of popcorn kernel.

Funding acknowledgement: Henan Innovation Project for University Prominent Research Talents (2005HANCET-12), Henan Scientific Technology Research Project (0623011700)

P197

QTL mapping for haploid male fertility in maize (Zea mays L.)

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Most maize haploid plants often display a high degree of sterility, but some of them perform different degree of fertility. The diploidization degree of female fertility is higher than that of male fertility if pollinated by diploids. The limited factor is resulted from whether or not the pollen is sterile or fertile. There is significant difference of male fertility for haploid plants among different maize germplasm. In our study, the inbred Zheng58 with low proportion of fertile haploids (FP) was crossed with inbred 8701 with high proportion of fertile haploids (FP). The F_1 hybrids 8701/Zheng58 was then induced with male inducer CAU5. Total of 5000 selected haploid kernels were used for QTL mapping of haploid male fertility. The haploid populations were identified of phenotype of male fertility and analyzed with 117 SSR markers in Hainan and Beijing. One QTL loci was found in one place, located at 3.07 bins. Other QTL loci were found in one haploid population originated from 8701/Zheng58 in both of two locations, located at 4.02 bins and at 10.02 bins, respectively. We also found that haploids male fertility was controlled by nucleus genes not cytoplasmic genes as there was no difference between reciprocal cross populations. We also found that haploids male fertility was a trait affected by multiple minor effect genes.

Funding acknowledgement: National Maize Industrial Technology System,National 863 Project

P198
Research for Autotetraploid Corn

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The author developed a method of receiving highly productive genotypes of tetraploid corn on the basis of complex studying and selection in population of genotypes by certain morphological and cytological criteria. On the basis of a selection material received as a result of the conducted researches the new grade of tetraploid corn included in the register of selection achievements of the Russian Federation was created.

P199
Selection at the level of haploid sporophyte as an efficient tool in maize improvement

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Within the last two decades, the efficiency of in-vivo production of maize haploids has significantly been improved facilitating the implementation of haploid technologies in maize breeding. Doubled haploid (DH) technology has already become an important tool in development of homozygous lines. However, the applied techniques of chromosome doubling still have some considerable disadvantages. Alternatively to the DH technology, pure haploid plants, due to their partial female fertility, can be used for some breeding and research purposes. In haploid plants as well as in DHs, allelic gene interaction (dominance and overdominance) is lacking making identification of genotypes with favorable additive gene effects a much easier task. This advantage was exploited in a recurrent selection scheme, Haploid Recurrent Selection (HRS), to improve the per se performance of two synthetic populations SA and SP. Selection for ear size and other agronomic traits carried out among haploid plants resulted in a relatively high gain per cycle (about 10%) for grain yield in the diploid populations. After five cycles of HRS, the grain yield of the SP population was improved up to the grain-yield level of F1 hybrids - 15 commercial hybrids used as checks. None of the check hybrids significantly exceeded the SPC5 population for ear length. For kernel row number, the SPC5 population was over the checks. Good performance of the SPC5 population in comparison with the commercial hybrids allowed us to conclude that selection at the level of haploid saprophyte may be a high-potential tool in maize improvement.

P200

Six-Year Experiences of Genetic Vulnerability of Maize Hybrids in China

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The 1/4 of world maize (approximately 35million ha) is grown in China and it is the second largest cultivation areas after USA. Average grain yield is about 65% of USA. Many factors including hybrids, soil management and climate have affected the yield difference. Since 1977, the author has helped hybrid maize breeding in China. In 2008, "Dr. Corn Seeds Co." for three North Eastern(NE) provinces with winter nursery in Hainan Province was started. Twelve plantings of breeding nurseries at four stations and observations in million farmers'fields had shown that genetic vulnerability (GV) of inbreeding of two outstanding hybrids, "Jeungdan958" and "Pioneer335" was found in advance. The first GV was from "JD958" by stem borers and smut casued by Ustilago maydis in 2009. It was cultivated on 10 million ha or over 60% of areas of total cultivation of NE. The second GV was from "Pioneer335" by root lodging and northern corn leaf blight caused by Exserohilum turcicum in 2012. It was cultivated on over 10 million ha or over 70% areas of the total cultivation of NE. Other potential GVs were seen from an insect and smuts in Harbin in 2013. Our company targets to breed only stable hybrids with genetically co-survival tolerance to major biotic and abiotic stresses. Maize grain yield in China may be increased by adoption of sustainable soil management by returning of stalk and leaves through mechanical harvest and animal manure application as well as by breeding and release of genetically diverse hybrids, not current copy-bred types. Registration of new hybrids by commercial companies and institutions not by government permit system is suggested. Results of maize performance and breeding progresses of outstanding and stable hybrids in China can affect world food, feed, industrial use and bio-energy production crises.

Funding acknowledgement: Dr. Corn Seeds Co., POSCO, HGU

P201

Sweet corn germplasm innovation for wine-making and its' application in breeding

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Since the introduction of sweet corn in China 1960s, varieties have been improved greatly, not only on production but on quality characters, sweet corn is becoming very popular for its sweetness, tenderness and palatability. Although there are over 267 thousand hectares sweet corn cultivated every year, its large scale cultivation has still been limited because of its short shelf-life especially of sweetness. It is urgent to make a breakthrough in its processed character. The great genetic diversity in color, fragrance or taste of sweet corn can provide vast basis for wine-making, but corn has high oil concentration, oil can produce fusel and has a negative influence to the wine taste if it's brewed directly. The bi-direction selection results to oil concentration in Illinois university has given us a good reminder for sweet corn germplasm innovation to oil concentration (Cathy C. Laurie et al., 2004).
In 2008, we begun to design a wine-making feature of sweet corn, small embryo kernels with low oil concentration have been selected from thousand of sweet corn material home and abroad, which was eventually selected in from a population of France, after be carefully propagated, it was crossed with several kinds sweet corn of big kernels, then a kind of recombinant of small embryo and long kernels has been selected from very large populations of their hybrid offspring , it's called the original populations for wine-making sweet corn, the oil concentration was about 3% in kernels, after 2 recycling selection, some inbred lines have been selected, their kernel type, cob type and plant type have be modified. Because of the addictive genetic effect which is mainly effect for oil concentration in kernel（Hui Li et al., 2013), a hybrid with low oil and high sugar concentration in kernels for high quality wine-making can be produced.

P202

A user-friendly sequence-indexed reverse genetics resource for maize

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A sequence-indexed reverse genetics resource is essential to fully exploit the maize genome sequence. Our NSF-PGRP-funded project is generating and sequence-indexing a collection of *Ds* transposon insertions using a cost-effective method that takes advantage of next-generation sequencing (NGS) technologies. Specifically, our goals for this project are: (1) Assemble a set of 120 roughly equidistant *Ds* * launching platforms carrying a *GFP* marker that allows simple visual selection of element transposition from any region of the genome and, thus, enable researchers to generate regional gene knock-out collections; (2) Sequence-index several thousand *Ds** insertion sites from dozens of model platforms by NGS of 3-dimensional DNA pools; and (3) Place all relevant information in our web-searchable database of insertion site sequences (*http://acdsinsertions.org*) cross-referenced to stocks available from the Maize Genetics Stock Center.
The following is a summary of our current progress. (1) Using a *C1* (colored seed) marker interrupted by a *GFP*-tagged *Ds** element, more than 150 *c1-m* transgenic lines with *Ds** transposition activity have been generated by *Agrobacterium* transformation and most of them have been mapped to the reference B73 genome. (2) More than 20,000 C' revertants bearing a *trDs** have been selected from lines with a high reversion frequency. In a test of 6,000 *C'* and *GFP* (purple, green fluorescent) selections from four different platforms, >90% were heritable, showing that the system is extremely efficient for recovering germinal *Ds** transpositions. By NGS of 3-D pools, more than 1,700 *trDs** target sites have been mapped to the reference genome with our publicly available pipeline InsertionMapper and others are presently being mapped. (3) All the lines generated in this project are listed in our web-searchable database, and, more than 1,200 of them have already been sent to the Maize Genetics Stock Center for distribution.

Funding acknowledgement: National Science Foundation (NSF)

P203

Activity and evolution of non-canonical Mutator transposons in maize

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The publicly-available UniformMu resource provides access to mutations in over 40% of maize genes, giving researchers invaluable new avenues to pursue reverse genetics in maize (see poster by Shan Wu). This resource, however, has thus far identified only mutations caused by the so-called "canonical" Mu transposons, Mu1 through Mu9 and Mu13 through Mu19. There are many related Mu elements that have not yet been mined in UniformMu material, and evidence indicates that these Mu's are actively transposing. Here we analyze the extent of transposition by these non-canonical, but closely-related, Mu elements in UniformMu, and discuss the feasibility using them to mine new mutants. Phylogentic analysis of 438 Mu terminal inverted repeats (TIRs) from the B73 genome revealed six major subclades of Mu elements, with conserved, but distinct, TIRs. We have adapted the Mu-seq protocol (used for identifying canonical Mu elements) for each subclade of non-canonical elements, and analyzed a subset of the UniformMu population to determine activity of these elements. Initial evidence indicates low levels of transposition by Mu's or most subgroups, and further analyses are underway. Even limited activity of these Mu elements could markedly increase their value to researchers pursuing forward-genetic approaches and to the overall potential for mapping Mu insetions in the UniformMu resource. In addition to the practical importance of non-canonical Mu's, the phylogentic analyses revealed their evolutionary relatedness and recent activity. Each of the six subclades included both homo- and hetero-morphic elements (designations based on the extent of similarity between "right" and "left" TIRs). Both types had clearly transposed in the genome, indicating previously unobserved interactions between Mu elements. In almost all instances, Mu element clades included MuDR-like sequences or their remnants. These findings add to our fundamental understanding of the Mu element family and further enhance our ability to utilize these elements.

Funding: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P204

Analysis of gene transcription and DNA methylation within the Dp3a neocentromere

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The centromere is the part of the chromosome that organizes the kinetochore, which is essential for the proper segregation and inheritance of genetic information. Neocentromeres originate occasionally from noncentromeric regions of chromosomes and have been found in many organisms. In Dp3a, the neocentromere was originally euchromatic and lacks CentC and CRM sequences, which are primarily present in native centromeres. We used RNA-Seq and Bisulfite-ChIP-Seq to analysis the transcription and DNA methylation of the neocentromere, and examined the underlying genes as to whether there is binding with CENH3 when they are actively transcribed. When we performed a detailed analysis of the 350 kb on the long arm of chromosome 3 that is covered by the neocentromere, bisulfite-ChIP-Seq results indicate a slightly increased DNA methylation level after centromere formation, similar to the methylation level of normal centromere regions, while CG methylation is increased and CHG methylation is lower. Collectively, these results may shed light on the mechanism of centromere formation.

Funding acknowledgement: National Science Foundation (NSF)

P205

Analysis of imprinted genes with developmental functions in the maize seed

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Genomic imprinting in plants is an epigenetic phenomenon by which a subset of genes is expressed in a parent-of-origin–dependent manner. Imprinted gene expression primarily occurs in the endosperm and is thought to influence seed size and embryo development. Although many maize imprinted genes have been identified through transcriptome analysis, imprinted genes with developmental functions in the maize seed have not been identified. We screened 178 *rough endosperm* (*rgh*) mutants for parent-of-origin effects using reciprocal crosses to inbred parents. Six *maternal rough endosperm* (*mre*) and three *paternal rough endosperm* (*pre*) mutants were identified: *mre1* , *mre*-21* , *mre*-40* , *mre*-217* ,*mre*-1014* , and *mre*-1147* as well as *pre*-58* , *pre*-144* and *pre*-949* . When inherited from the female parent, *mre* seeds show a rough, etched, or pitted endosperm surface as well as a reduced seed size and weight. The *pre* mutants show the converse inheritance pattern with *pre* pollen conferring a seed phenotype after fertilizing wild-type ovules. Preliminary characterization of the *mre* and *pre* isolates shows a range of seed defects with several mutants showing embryo defects in addition to the endosperm phenotype. Molecular mapping experiments have identified one locus, *mre1* , on chromosome 4, and the *mre1* locus shows endosperm development delay. RNA-seq is being used to analyze imprinted gene expression in *mre1* seeds.

Funding acknowledgement: United States Department of Agriculture (USDA)

P206

Changes in DNA methylation profile in maize under herbicide stress conditions

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DNA methylation has been shown to be involved in gene silencing at both transcriptional and posttranscriptional levels. Transcriptional gene silencing is associated with hypermethylation of promoter sequences, while post-transcriptional gene silencing is linked with hypermethylation of transcribed or coding sequences. In the plant genome, methylated cytosine residues (m5C) are found in three nucleotide-sequence contexts: symmetrical CG, and two categories of non-CG sites, symmetrical CNG and asymmetrical CNN sites (where N is A, T or C). DNA methylation (both asymmetric and symmetric) plays a crucial role in the regulation of gene expression, in the activity of transposable elements, in the defense against foreign DNA, and even in the inheritance of specific gene expression patterns. The link between stress exposure and sequence-specific changes in DNA methylation was hypothetical until recently, when it was shown that stresses can induce changes in gene expression through hypomethylation or hypermethylation of DNA.

It has been observed in the fields that some maize lines display higher resistance to herbicides than others but to this day the molecular mechanisms of such resistance remain unknown. To detect changes in DNA methylation under herbicide stress in two maize lines displaying different susceptibility to RoundUp® we used Methylation Sensitive Amplified Polymorphism (MSAP). It is a technique where isoschizomers Hpa II and Msp I are used to determine the differences in DNA methylation due to enzyme's differential sensitivity to DNA methylation. We observed differences in methylation profiles between the two tested lines. Differentiating DNA bands were eluted and sequenced. Analyses of 197 DNA fragments using Blastn and Maize GDB databases allowed us to divide them into several groups representing genes encoding for transporter proteins, transferases, methyltransferases, genes involved in stress responses but also transposons.

Funding acknowledgement: National Science Centre, Poland

P207

Characterization of miR169 and NF-YA families and their expression pattern responding to abiotic stresses in maize

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The miR169 family is a large and conserved miRNA family in many plant species, targeting NF-YAs, a subunit of NF-Y transcription factors. Previous studies have identified some miR169/NF-YA modules are important regulators for plant developmental and stress-induced responses. To understand the roles of miR169/NFYAs responding to abiotic stressed in maize, miR169 and NFYA families were analyzed in this study. Zm-NFYA family consists of 14 members with a conserved domain. MiR169 family comprises 18 members in maize, generating 10 mature products, which target seven of fourteen ZmNF-YA genes. The seven ZmNF-YAs all located in nuclei, have not transcriptional activity alone. We investigated the expression pattern of the miR169 and NF-YA families under drought (PEG), abscisic acid (ABA) and salt (NaCl) stresses in roots. The data showed that zma-miR169 family members generally were dramatically down-regulated by short-term (i.e. 0~48h) and increased upon a long-term (i.e. 15d) under three abiotics stresses.We also showed that part ZmNF-YA family members demonstrating a reverse correlation with the expression of zma-miR169s. Interestingly, ZmNF-YA14 is found to be regulated by most zma-miR169 members and participating in three abiotic stresses response. Furthermore, overexpression ZmNF-YA14 transgenic lines have shorter root length and increase the salt tolerance of maize. Finally, we found that zma-miR169s/ZmNF-YA14 may involve in root elongation responding to the three abiotics stresses.

Funding acknowledgement: Ministry of Science and Technology

P208

Characterization of the role of duplicated *ZmPho1;2* genes in maize phosphate homeostasis

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The PHO1 protein of *Arabidopsis* has been characterized to play a key role in both phosphate transport and sensing of phosphate status (Hamburger et al., 2002). Furthermore, recent results suggest PHO1 as a potential target for enhancement of internal phosphate use efficiency. In maize, we have identified two co-orthologs of the *Arabidopsis PHO1* gene (*ZmPho1;2a* and *ZmPho1;2b*) that we hypothesise to represent a paralogous pair retained following whole genome duplication. Genome-scale analyses suggest that retention of both paralogs following genome duplication is not typical (Schnable et al., 2011). As such, the presence of *ZmPho1;2a* and *ZmPho1;2b* in the genome may indicate incomplete fractionation as part of an ongoing process of gene-loss, or, alternatively, suggest the action of selective forces working to maintain both genes. Significantly, studies in *Arabidopsis* indicate that changes in PHO1 dosage have an important impact on plant phosphate homeostasis (Rouached et al., 2011).

To initiate functional characterization of maize *Pho1* genes, and specifically to investigate functional divergence, we have used the *Activator* (*Ac*) and *Dissociation* (*Ds*) transposon system as a reverse genetic tool to identify novel insertions in *ZmPho1;2a* and *ZmPho1;2b*.
A *Ds* element was identified in an intron of *ZmPho1;2b*, and re-mobilized to generate additional insertions, including one identified in the 5' UTR of the gene. An Ac element was identified 650kb upstream of *ZmPho1;2a*, and remobilized to generate a novel insertion into the sixth exon of the gene. Subsequently, we have identified a number of stable footprint derivatives from this *Ac* insertion. We will present analysis of these novel transposon-mediated events and an initial characterization of *Zmpho1* mutant plants.

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Funding acknowledgement: We thank CINVESTAV (Centro de Investigacion y de Estudios Avanzados del Instituto Politecnico Nacional), the Mexican Consejo Nacional de Ciencia y Tecnologia (CONACyT), and the National Science Foundation (NSF) for funding of this work.

P209

Comprehensive analysis of imprinted genes in maize reveals allelic variation for imprinting and limited conservation with other species

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In plants, a subset of genes exhibit imprinting in endosperm tissue such that expression is primarily from the maternal or paternal allele. Imprinting may arise as a consequence of mechanisms for silencing of transposons during reproduction, and in some cases imprinted expression of particular genes may provide a selective advantage such that it is conserved across species. Separate mechanisms for the origin of imprinted expression patterns and maintenance of these patterns may result in substantial variation in the targets of imprinting in different species. Deep sequencing of RNAs isolated from reciprocal crosses of four diverse maize genotypes provided a comprehensive analysis that allows evaluation of imprinting at more than 95% of endosperm-expressed genes. We find that over 500 genes exhibit statistically significant parent-of-origin effects in maize endosperm tissue, but focused our analyses on a subset of these genes that had >90% expression from the maternal allele (69 genes) or from the paternal allele (108 genes) in at least one reciprocal cross. Over 10% of imprinted genes show evidence of allelic variation for imprinting. A comparison of imprinting in maize and rice reveals that 13% of genes with syntenic orthologs in both species exhibit conserved imprinting. Genes that exhibit conserved imprinting between maize and rice have elevated nonsynonymous to synonymous ratios compared to other imprinted genes, suggesting a history of more rapid evolution. Together, these data suggest that imprinting only has functional relevance at a subset of loci that currently exhibit imprinting in maize. Additionally, these data can be used to investigate potential mechanism(s) for silencing imprinted alleles and study possible phenotypic roles of imprinted genes.

Funding acknowledgement: National Science Foundation (NSF)

P210

De novo small RNA loci annotation in maize leaves and their expression changes in response to environmental stresses

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Plants cope with environmental changes by modulating protein-coding gene expression and also by microRNA regulation. Stress-dependent miRNA expression changes are important for the regulation of plant growth and development. Abiotic stresses also influence transposable elements (TEs), releasing their silencing. Since TEs can affect the regulation of genes, they may play important roles in plant development and adaptation. Heterochromatic short-interfering RNAs (hc-siRNAs) act in the RNA-directed DNA methylation (RdDM) epigenetic pathway that suppresses transposons activity. Studying hc-siRNA expression alterations following abiotic stresses is therefore helpful in understanding how RdDM is involved in the plant response and adaptation to environmental changes. We applied drought, salt, and the combination of two stresses to maize wild-type (B73) and *rmr6* mutants (*required to maintain repression 6*) V5-V6 plants and collected the youngest wrapped leaf after 10 days of treatment, then after 7 days of recovery period, for every stress conditions and the control. Illumina small RNA (sRNA) sequencing was performed on the 48 samples; on a subset of samples total RNA sequencing and ChIP sequencing (H3K4me3, H3K9ac, H3K27me3) were also performed. Based on analysis of these data, we de novo annotated 188,938 sRNA loci. 143 are microRNA loci: 70 are known loci, only half of them show the same mature sequences annotated in miRBase, suggesting that the preferential processing of the hairpin precursor can varies between different kind of samples; 68 give rise to putative novel microRNAs. We are studying their response to the stresses and identifying their targets. About 10% of the total annotated sRNA loci may be hairpin-derived, while the majority are likely hc-siRNAs with double-stranded precursors. We are analyzing their genome distribution and the stress effects on their expression. By the comparison with total RNA sequencing data, we are seeing if the annotated small RNA loci are expressed among the long non-coding RNAs. Mutants allow understanding what sRNAs are Pol IV-dependent and observe sRNAs different behavior under control and stress conditions when RdDM is impaired.

P211

Epigenetic variation through the breeding processes of maize

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Maize grain yield has been remarkably improved during modern maize breeding over the last four decades, but the concurrent epigenetic changes during this period remain unknown. Here, we performed MethylC-seq and RNA-seq on 4 related inbred lines with known pedigree information. DNA methylation show instability with frequency of 10^{-6}-10^{-5} per cytosine site per year, several orders of magnitude higher than that of DNA mutations. A large fraction of epipolymorphisms in both CG and CHG contexts occur in clusters with median size of 222 bp (CG) and 338 bp (CHG). Characterization of the sequence properties of the epimutable sites suggests they are primarily targets of DRM 1/2 in the RNA dependent DNA methylation pathway and that sequence composition is an important factor in the stability of DNA methylation. Half of the sites that epimutated during the breeding process existed as epipolymorphisms between the parental lines, indicating a paramutation-like process may occur in regions with a heterozygous DNA methylation state. Furthermore, in identical-by-descent regions of the genome, a preponderance of genic methylation polymorphisms are associated with changes in gene expression in seedling shoot tissue, and presumably phenotype. Analysis of epigenetic changes over the course of historical maize breeding is a valuable new avenue in the exploration for crop improvement. These data lead us to suggest that novel epihaplotypes, in addition to DNA variation, are a substrate of selection during breeding, and that epigenetic variation between parents may also contribute to heterosis in hybrids.

Funding acknowledgement: National Science Foundation (NSF), National Basic Research Program (973 program), 863 Project

P212

Epigenetics regulation of maize transcriptome: from coding, *via* TEs, to long non-coding RNAs and beyond

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The plastic adaptability of plants to environmental changes involves sophisticated responses of cellular physiology, gene regulation and genome remodelling. Increasing experimental evidences suggest a predominant role for epigenetic mechanisms, such as DNA methylation, chromatin modifications and non coding RNAs, in abiotic stress responses. Stress-induced epigenetic release may also result in genome destabilization, with the activation and/or the transcription of DNA transposons and retroelements usually silenced which may result in novel gene expression and DNA rearrangements.

We used a total RNA-Seq strategy to deeply analyze the leaf transcriptome of both B73 and epiregulator mutant *Required to Maintain Repression6* (*rmr6*; involved in siRNA biogenesis and in the RNA-directed DNA Methylation pathway) after drought and salt stress application. Our reference annotation based transcript (RABT) assembly approach allowed to identify, in addition to the 110,191 loci annotated in the Zea_mays.AGPv3.20 genome release, 21,471 potential novel isoforms and 3,406 new transcribed loci mapping in intergenic regions. Interestingly we also found 447 transcripts matching on the opposite strand of annotated loci, which represent potential antisense transcripts.

We are now assessing the protein-coding potential of all these new classes of transcribed loci, analyzing their expression modulation as effect of the stress application in B73 and *rmr6* mutant leaves.
Preliminary results indicate that the main part of newly identified intergenic transcripts correspond to transposable elements exclusively expressed in *rmr6* mutant and upregulated by the stresses application. Analysis of smallRNA populations from these samples will allow also to determine the role of PolIV-derived siRNAs in TEs and intergenic transcriptional silencing under control and stressing conditions building a genome-wide survey of epigenetic regulation of genome transcription and stability.

P213

Genetic and epigenetic regulation of response to drought and salt stress in maize

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Plants commonly adapt to biotic and abiotic stresses by regulating expression levels of thousands of stress-related genes. This modulation of gene expression patterns is, at least in part, achieved by epigenetic regulation mechanisms and an increasing number of studies have shown that histone modifications play a key role in gene expression and plant development under stress.However, genome-wide surveys of such stress-related modifications are very limited, especially for crops.

We applied drought, salt, and drought plus salt mild stresses to maize wild-type (B73) V5-V6 plants and collected the youngest wrapped leaf after 10 days of treatment and after 7 days of recovery. For every stress condition and the control sample we analyzed gene expression changes by total RNA-Seq. Therefore, on control and drought stressed plants at both timepoints, the "genetic" regulation of stress response was then correlated with the "epigenetic" counterpart by using ChIP-Seq, to investigate the genome-wide distribution pattern of histone H3 lysine4 trimethylation (H3K4me3), histone H3 lysine9 acetylation (H3K9ac), histone H3 lysine27 trimethylation (H3K27me3). ChIP-seq analysis of the three histone modifications are revealing that the stress treatment altered the epigenomic landscape and we are currently evaluating the entity of these changes in the leaf treated tissues. Indeed different modifications varied in number and direction. Digital gene expression analysis coupled with Gene Ontology enrichment analysis revealed the modulation of many stress-related genes and a reference annotation based transcript (RABT) assembly approach allowed the identification of new splicing variants and novel transcribed intergenic regions. This implemented transcriptome annotation will be useful to correlate the genome-wide distribution and enrichment of each histone marks with transcription modulation and activation in response to stress, in particular for H3K27me3 which plays a critical role in facultative heterochromatin establishment, a repressive chromatin status normally associated with developmental and stress-related regulation of gene expression.

P214

Genome-wide analysis of terminal-repeat retrotransposons in miniature (TRIM) in maize

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Long terminal repeat (LTR) retrotransposons dominate the genetic capacity in plants, where they may compose up to 80% of the genome. They are important sources of genetic and phenotypic diversity and can impact gene and genome evolution. We identified a TRIM (terminal-repeat retrotransposons in miniature) in maize genome, which is a group of small nonautonomous LTR retrotransposon. It has 532bp in length, and contains 232bp LTR, A total of approximately two hundred copies were identified in the maize genome B73, which were dispersed on all 10 chromosomes. These TRIMs share high sequence similarity, and one copy has two identical LTRs, which suggested that they may still be active. Since TRIMs have no coding capacity, retrotransposition must depend on their autonomous counterparts. We then identified an autonomous retrotrsansposon that share high sequence similarity with the TRIMs, and has been verified to be active in maize pollen by RT-PCR. Due to the potential activity, TRIMs may provide a valuable tool for gene tagging system and molecular marker in maize.

Funding acknowledgement: National Science Foundation (NSF)

P215

Guide to Maize Mutant Phenotypes (http://mutants.maizegdb.org)

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Our online Guide to Maize Mutant Phenotypes is now available, presenting many mutant phenotypes photographed and described by colleagues and myself (MGN) in 65 years as a maize geneticist. Currently, 829 high-quality images, representing 352 phenotypes, have been selected to display, the diversity of mutants in maize. Each phenotype has a brief definition and one good representative image, or in some cases (virescent, lesion) multiple images demonstrating the range of expression. Captions describe each picture in detail, and in some cases include information such as effects of background, temperature, etc. The Guide is intended for a broad audience, from a beginning student to established researchers wanting to compare an interesting new plant variation with those previously described. We invite your suggestions and additional images and data to help improve and expand this resource. A documentary form for submitting such additions will be provided. The Informatics Research Core Facility at the University of Missouri (Scott Givan, Associate Director) is providing informatics and programming support for my own "Mutant Database" from which this data was derived, and for generating the web pages for our Guide from it. We are grateful to MaizeGDB for hosting
the Guide and for the generous donation of their time and services. Support from NSF grant IOS- 1239861 for current work is gratefully acknowledged. We thank NSF, USDA, and Pioneer Hi-Bred for many years of valuable support.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P216

Rapid invasion of euchromatic regions by centromeric retrotransposons at sites of neocentromere formation in maize

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Maize centromere 5 (CEN5) is a neocentromere that has formed over one of three position: CEN5M represents the original maize CEN5, which was translocated from the corresponding sorghum centromere into a euchromatic region by sequential hemicentric inversions and contains the tandem centromeric CentC repeats typical for most maize centromeres. Upon loss of the CentC cluster at CEN5M, a neocentromere forms several megabases upstream (CEN5L) or downstream (CEN5R) of CEN5M, on either entirely, or mostly, euchromatic DNA. Using ChIP-qPCR we determined that of the 25 NAM line parents, 13 use CEN5L and 10 use CEN5R. Survey sequence from the NAM line parents revealed frequent large deletions and several recombination breakpoints within or near CEN5. To obtain accurate divergence time estimates for the CEN5 regions of the different NAM lines, we generated phylogenetic trees based on SNPs from an approximately 7 Mb region in which we detected no evidence of recombination. The phylogenetic reconstruction is confirmed by shared retrotransposon insertions and lineage-specific deletions, and revealed that the shift from CEN5M to either CEN5L or CEN5R has occurred at least four independent times since maize domestication, and is associated with novel centromeric retrotransposon (CR) insertions in the overwhelming majority of cases. Our analysis has revealed a much higher than expected frequency of CR element insertions into neocentromeres, and provides further support for our hypothesis that CR elements play a functional role in centromeres.

Funding acknowledgement: National Science Foundation (NSF), University of Hawaii

P217

Selective acquisition and retention of genomic sequences by Pack-MULEs in grasses

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The process of gene duplication followed by sequence and functional divergence is important for the generation of new genes. Pack-MULEs, nonautonomous Mutator-like elements (MULEs) that carry genic sequence(s), are potentially involved in generating new open reading frames and regulating parental gene expression. These elements are identified in many plant genomes and are most abundant in rice (Oryza sativa). Despite the abundance of Pack-MULEs, the mechanism by which parental genes are captured by Pack-MULEs remains largely unknown. In this study, we identified all MULEs in rice and examined factors likely important for sequence acquisition. Terminal inverted repeat MULEs are the predominant MULE type and account for the majority of the Pack-MULEs. In addition to genic sequences, rice MULEs capture guanine-cytosine (GC)-rich intergenic sequences, albeit at a much lower frequency. MULEs carrying nontransposon sequences have longer terminal inverted repeats and higher GC content in terminal and subterminal regions. An overrepresentation of genes with known functions and genes with orthologs among parental genes of Pack-MULEs is observed in rice, maize (Zea mays), and Arabidopsis (Arabidopsis thaliana), suggesting preferential acquisition for bona fide genes by these elements. Pack-MULEs selectively acquire/retain parental sequences through a combined effect of GC content and breadth of expression, with GC content playing a stronger role. Increased GC content and number of tissues with detectable expression result in higher chances of a gene being acquired by Pack-MULEs. Such selective acquisition/retention provides these elements greater chances of carrying functional sequences that may provide new genetic resources for the evolution of new genes or the modification of existing genes.

Funding acknowledgement: National Science Foundation (NSF)

P218

Small RNA profiling revealed the maize *Ufo1*-modulated epigenetic regulation via 24 nt small RNA in tissue-specific manner

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pericarp color 1 (p1) regulates flavonoid biosynthetic genes which produce brick-red phlobaphene in maize. Many *P1* alleles such as *P1-wr* (white pericarp, red cob) exhibit tissue-specific pigmentation as the result of their differential expression patterns. *Unstable factor for orange 1 (Ufo1)* is a dominant modifier which regulates the expression levels of certain *p1* alleles epigenetically. Compared with the colorless pericarp in *P1-wr* plants, *P1-wr; Ufo1-1* plants exhibit enhanced pigmentation in various tissues, which is associated with hypomethylation of *P1-wr* allele and increased level of *p1* expression. To investigate whether *Ufo1* function is associated with small RNA (sRNA)-directed epigenetic regulation, we conducted extensive sRNA profilings in leaf, tassel, young ear, and pericarp of *P1-wr; Ufo1-1* plants. Our results showed a 1.5 fold reduction in the 24nt abundance only in the *Ufo1-1* pericarp. *Ufo1* impacted sRNA abundance in a tissue-specific manner: while 4 times more sRNA-generating loci were repressed (5,982) than induced (1,547) in*Ufo1-1* pericarp, more sRNA clusters were induced than repressed in *Ufo1-1* young tassel or young ear. Further analysis revealed that most *Ufo1*-repressed clusters were regions generating 24nt sRNA. Interestingly, in a silenced *Ufo1-1* homozygous plant (genotyped homozygously for *Ufo1-1* mutation but lost the phenotype of enhanced pigmentation), the 24nt abundance in pericarp was similar to the wildtype level. Furthermore, the number of repressed sRNA clusters (1,311) in silenced *Ufo1-1* pericarp was greatly reduced than that in pericarp of *Ufo1* expressor, suggesting that the increase of 24 nt silencing sRNA may contribute to the regain of transcriptional repression of genomic loci including *P1-wr*allele in silenced *Ufo1-1* pericarp. Our results indicated that *Ufo1* affects sRNA especially 24 nt abundance in a locus-specific rather than a global fashion. *Ufo1* may function in the maintenance of chromatin and DNA methylation state in a tissue-specific manner.

Funding acknowledgement: National Science Foundation (NSF)

P219
Specific tandem repeats are sufficient for paramutation-induced trans-generational silencing

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Paramutation is a well-studied epigenetic phenomenon in which *trans* communication between two different alleles leads to meiotically heritable transcriptional silencing of one of the alleles. With paramutation at the *b1* gene, a regulatory gene of the maize pigmentation pathway, the low expressed *B'* epiallele imposes its low transcription rate onto the high expressed *B-I* epiallele *in trans*, thereby changing *B-I* into *B'* in a mitotically and meiotically heritable manner. This change occurs with a 100% frequency. Seven tandem repeats of a 853 bp sequence, 100-kb upstream of the *b1* gene are required for the *in trans* interaction and for high *b1* expression. The *B'* repeats are DNA hypermethylated compared to the*B-I* repeats. The genes required for paramutation indicate a role for heterochromatic siRNAs, but as both the *B'* and the *B-I* repeats are transcribed and result in the production of siRNAs, siRNAs may not be sufficient.

A transgenic approach showed that paramutation can be mediated by transgenic *b1* tandem repeat sequences, also by tandem repeats consisting of only one half of the repeat unit, demonstrating that these sequences are sufficient for paramutation and that an allelic position is not required for paramutation. Tandem repeats of the other half of the repeat unit did not mediate paramutation, indicating specific tandem repeats are required. Transgenic tandem repeats increased the expression of a reporter gene in maize, demonstrating the repeats contain transcriptional regulatory sequences. Transgene-mediated paramutation required the *mediator of paramutation 1* gene, which is necessary for endogenous paramutation, suggesting endogenous and transgene-mediated paramutation both require the RNA-mediated transcriptional silencing pathway. While all tested repeat transgenes produced siRNAs, not all transgenes induced paramutation suggesting that, as with endogenous alleles, siRNA production is not sufficient for paramutation. The transgenic repeats did not show the extensive DNA methylation level as observed for the *B'* repeats and the repeat transgene-induced silencing was less efficiently transmitted than silencing induced by the repeats in endogenous *b1* alleles, suggesting that the strength of the repeat transgene-induced silencing correlates with the DNA methylation level at the repeats.

Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF), CNPq, KNAW

P220

ZmAGo18a, a meiosis-enriched Argonaute, is required for inflorescence development and epigenomic reprogramming of the male gametophyte in maize

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Argonautes (AGOs), the central protein component in small RNA silencing pathways, are pivotal in regulation of gene expression. However, biological function of AGOs in maize (Zea mays L.) remains largely unexplored. In the present study, total of 17 AGO genes of maize (ZmAGOs) were identified by a Hidden Markov Model and were validated by rapid amplification of cDNA end assay. Subsequently, quantitative PCR revealed that expressions of these genes were higher in reproductive than vegetative tissues, and presented four temporal and spatial expression patterns which were modulated by 5′-untranslated exon, DNA methylation and microRNA-mediated feedback loop. Intriguingly, ZmAGO18b was highly expressed in tassels during meiosis. Both RNA in situ hybridization and immunofluorescence showed that ZmAGO18b was enriched in tapetum and germ cells in meiotic anthers. RNA Immunoprecipitation revealed ZmAGO18b bound Copia and Gypsy-derived 24nt small RNAs, and both zma-miR319 and zma-miR172 family 21nt micorRNAs selectively in meiotic tassels. In addition, a mutant caused by a Mutator insertion in the first exon was identified. RNA-seq validated that ZmAGO18b expression was markedly down-regaluted in Mutant tassels, and gene expressions including known targets of miR319 and miR172, those genes involved in miR319 and miR172 regulated pathways, and inflorescence development associated genes, were significantly altered in the mutant tassels. Bisulfite sequencing showed that methylation of non-CpG sequence context of 180-bp knob DNA in meiotic anthers and CpG context of 350-bp knob DNA in pollen was significantly lower in the mutant than in wild type. This suggested that ZmAGO18b is a tapetum and germ cell-specific member of AGO family, and was likely involved in the regulation of inflorescence development mediated by miR172 and miR319, and epigenomic reprogramming of male gametophytes by the 24nt RNA-directed DNA methylation mechanism in maize.

Funding acknowledgement: The work was supported by the National Hi-Tech program of China (www.863.gov.cn) (2012AA10A307), the National Natural Science Foundation of China (www.nsfc.gov.cn)(31071429)

P221

RNA Binding Protein 48 (RBM48) is Critical for Maize Endosperm Development and Plant Viability

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The precise recognition and removal of introns from pre-mRNA is a fundamental process required for the expression of eukaryotic genes. However it is poorly understood in plants due to a lack of *in vitro* splicing assay. The arginine/serine-rich (SR) family of highly conserved phosphoproteins plays an important role in splice site selection during both constitutive and alternative splicing. We mapped a UniformMu, *rough endosperm (rgh)* seed mutant with severe grain-fill and embryo development defects. Sequencing of *Mutator* Flanking Sequence Tags (*Mu* FSTs) identified an insertion in an SR-related protein that co-segregates with the *rgh* phenotype. The SR-related protein is orthologous of an uncharacterized human protein annotated as RNA binding protein 48 (RBM48). RT-PCR identified four different alternatively spliced transcript isoforms from the normal *Rbm48* allele but failed to detect any transcript from the mutant *rbm48-1* allele. Public UniformMu reverse genetics identified an independent insertion, *rbm48-2*. Both alleles segregate for the same *rgh* phenotype and fail to complement when *rbm48-1*/+ is crossed by *rbm48-2*/+. These data indicate that *rbm48* causes the seed phenotype. We conclude that RBM48 has an integral role in the RNA splicing network required for seed development and grain-fill.

Funding acknowledgement: National Science Foundation (NSF)

Late Poster Abstracts

P222

Transposon-based mutagenesis of the maize gene *Ixbalanque* (*Ixb*), a homolog of the symbiotic potassium channel *POLLUX*

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Establishment of arbuscular mycorrhizal (AM) and rhizobial symbioses in legumes requires the action of a number of shared signalling components constituting the so-called "common symbiosis pathway" (SYM). It had been hypothesised that the pathway first evolved along with the mycorrhizal association, the older of the two symbioses. Indeed, it has been shown that the genes encoding the SYM components are conserved in rice and that mutation of these genes disrupts AM symbiosis. To date, there has been no functional characterization of SYM genes in maize. To initiate the molecular characterization of maize AM interactions, we have identified a maize homolog of the SYM component *POLLUX/DMI1*, a gene encoding a nuclear ion channel implicated in calcium spiking events during pre-symbiotic signalling. By analogy to *POLLUX*, we have named this gene *Ixbalanque* (*Ixb*) for one of the hero twins of Mayan mythology. In common with rice *POLLUX*, *Ixb* is a single-copy gene, and we have selected it as a target for gene tagging using the *Ac/Ds* system. We have mobilized a *Ds* donor 57.7 kb upstream of the predicted *Ixb* transcriptional start site. Using a pooling strategy, 4,160 test-cross progeny have been screened, and three putative germinal insertions identified. We are now in the process of recovering these events prior to phenotypic analysis. Identification of mutant alleles of maize SYM genes will open the door to molecular dissection of AM symbioses in maize, allow investigation of the role played by the SYM pathway in other mutualistic and pathogenic interactions, and provide valuable material for the direct estimate of AM benefit in a field setting.

Funding acknowledgement: CONACYT

P223

Genetic variation of symbiosis-associated phosphate transporter (PHT1) genes in maize

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The inherent genetic diversity of maize provides a powerful material for allele mining associated with traits of interest (Buckler et al., Curr Op Plant Biol 9, 172, 2006). Screening the 26 founder lines of the maize Nested Associated Mapping (NAM) population identified candidate lines with significant variation in arbuscular mycorrhizal (AM) responsiveness and additionally lines with contrasting tolerance to low Pi-fertilization.

Variation in either Pi uptake efficiency (PAE) or the internal Pi use efficiency (PUE) may correlate with either phenotypic variation. The uptake of Pi across the plasma membrane is typically mediated by Pi transporter proteins belonging to the PHT1 class. Variation in functionality or expression levels of PHT1 genes therefore might underpin variation in PAE and/or PUE. Here, we determined the sequence variation of *PHT1* genes across the maize diversity panel. Focusing on the subset of candidate lines the transcriptional response of selected *PHT1* genes to different phosphate regimes and/or AM colonization was examined. The molecular data were correlated with shoot biomass production and symbiotic phosphate uptake.

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The maize brown midrib4 (bm4) gene contributes to one-carbon metabolism, which is required for normal lignin biosynthesis.

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Consistent with earlier studies, we found that maize *brown midrib 4 (bm4)* mutants exhibit a modest decrease in lignin concentration and an overall increase in the S:G lignin ratio relative to wild-type. Fine mapping analysis of *bm4* narrowed the candidate region to an ~105kb interval on chromosome 9 that contains six genes. One of these genes was significantly down-regulated in an RNA-Seq experiment that compared expression between the *bm4-ref* allele vs. non-mutant siblings. Seq-Walking analyses detected unique *Mu* insertions in this down-regulated gene in stocks that carried 4/10 *bm4-Mu a*lleles. The *bm4* gene contributes to one carbon (C1) metabolism, demonstrating the importance of this pathway to normal lignin biosynthesis.

Funding acknowledgement: National Science Foundation (NSF)

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Development of a new statistical method to detect CNV using micro-array data: Application to a diversity panel of 336 inbred lines using 50K maize infinium HD

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Copy Number Variants (CNV) are widespread in Maize but their distribution along the genome and germplasm remains largely unknown. We developed a new statistical methods based on hidden Markov models to detect CNV in a large set of individuals using micro-array fluorescence. This new method takes into account simultaneously dependency between loci due to their physical linkage on chromosomes and between inbred lines due to their relatedness. The CNV detection should be improved by taking into account the kinship matrix as relatedness between lines measure the mean probability to identical by descent at any locus.

We use a multivariate hidden Markov models to take into account several types of correlated hidden processes at the same time. Since maximum likelihood inference becomes intractable when the individuals become large, we propose an approximate inference algorithm based on a variational approach to infer the structural variations in plant genomes, resulting in a variational EM (VEM) algorithm.

The new method is applied to detect CNV at 55 535 locus genotyped by maize Illumine Infinium HD array on a diversity panel of 336 inbred lines. In experiment, the kinship matrix is composed of correlation coefficient among all lines. We discovered 430 deleted segments larger than 500kbp, and 64 deleted segments larger than 1Mbp which the most segments is distributed on chromosome 6. Moreover, in chromosome 6, we find that there are 124 lines which share a deleted segment with length of 619301. This method may be extended in the experiment CGH.

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National Science Foundation
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We thank these contributors for their generosity!