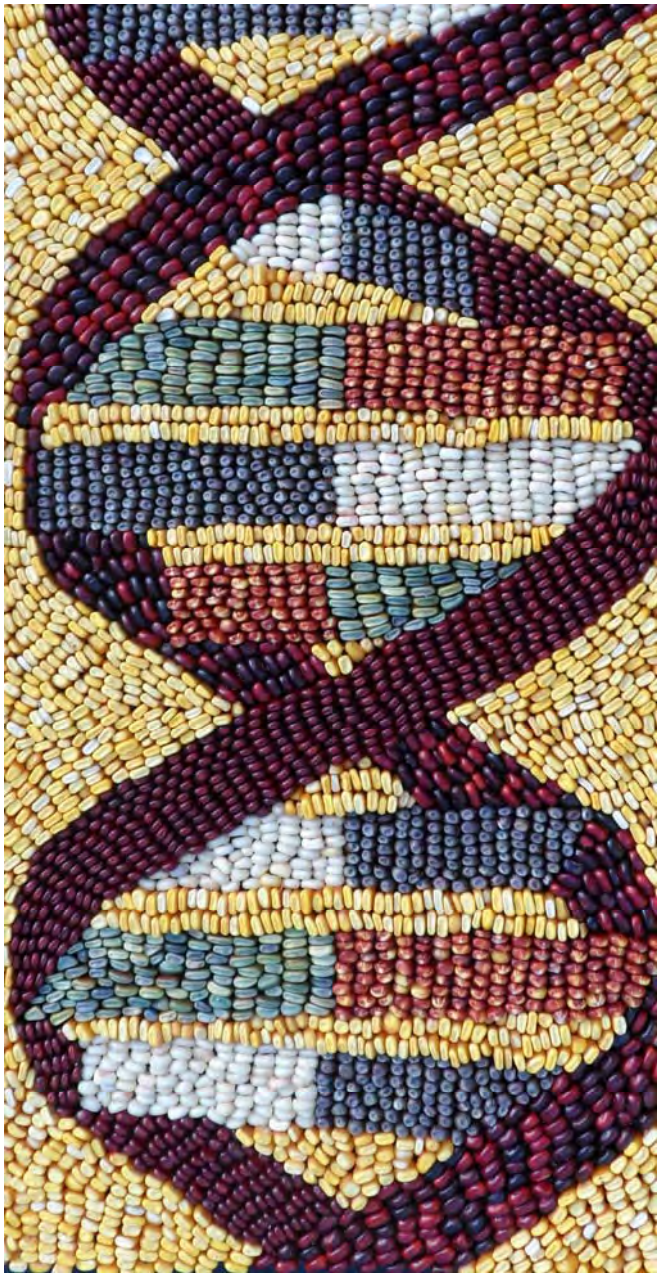
57th Annual Maize Genetics Conference

Program and Abstracts



March 12 – March 15, 2015

Pheasant Run, St. Charles, Illinois

This conference received financial support from:

National Science Foundation
DuPont Pioneer
Monsanto
BASF Plant Science
Syngenta
Dow AgroSciences
KWS
Biogemma
KeyGene
AgReliant

We thank these contributors for their generosity!

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Cover image description

This Maize DNA Mosaic depicts a stretch of DNA in the maize *r1* gene, which codes for red color in the kernel aleurone. It is assembled from seven different colors of maize harvested from research fields in upstate New York and was inspired by the (much larger) maize mosaics at The Corn Palace (Mitchell, South Dakota).

Cover art by

Jason Wallace
Cornell University

General Information

Meeting Registration

Thursday: 3:00 to 8:30 PM: There will be a table in the Solarium (near main lobby).
9:00 PM to 10:00 PM: There will be a table in the Mega Center.
Friday: 7:00AM - 8:15AM: There will be a table in the Mega Center.

Meals

All meals will be served buffet style in the Mega Center; 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 the St. Charles Ballroom.

Posters will be presented in the Mega Center, adjacent to where we will have meals. 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 1:30-3:00 PM on Friday and 3:00-4:30 PM on Saturday. Presenters of even numbered posters should stand by their posters 3:00-4:30 PM on Friday and 1:30-3:00 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The maize meeting is a forum for presentation and discussion of unpublished material.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.

Hospitality

After the evening sessions on Thursday and Friday there will be informal socializing and poster gazing in the Mega Center, with refreshments provided until 1 AM. On Saturday evening there will be informal socializing in the Mega Center, with music, dancing and refreshments until 2 AM.

After 1 AM, a double suite on the 16th floor of the Tower (rooms 1611-1613) is available for continued socializing. This is a "private party room" and alcoholic beverages may be brought in; however, you must stay in this room if you are carrying drinks and dispose of trash and bottles in the party room.

Steering Committee

Please share your suggestions and comments about the meeting with the 2015 Steering Committee

Mark Settles, Chair ..... (settles@ufl.edu)
David Braun, co-Chair ..... (braundm@missouri.edu)
Wes Bruce ..... (wes.bruce@basf.com)
Sherry Flint-Garcia ..... (flint-garcias@missouri.edu)
Jinsheng Lai ..... (jlai@cau.edu.cn)
Milena Ouzunova..... (m.ouzunova@kws.com)
Gernot Presting ..... (gernot@hawaii.edu)
Ruairidh Sawers..... (rsawers@langebio.cinvestav.mx)
Ann Stapleton ..... (stapletona@uncw.edu)
Petra Wolters..... (petra.wolters@cgr.dupont.com)
Amanda Wright..... (amanda.wright@unt.edu)

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

Acknowledgements

Many thanks go to Carson Andorf 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 Margy Moore and the Pheasant Run staff for their help in organizing this conference, and to Darwin Campbell and John Portwood for providing AV and other support. Thanks go to Mark Settles, David Braun, Wes Bruce, and Milena Ouzunova for their efforts in securing funding to support graduate student attendance at this meeting. Finally, many thanks go to Marty Sachs for his work as local organizer and his wisdom in all things related to the Maize Meeting.

Useful Links

2015 Maize Meeting Website

http://maizegdb.org/maize_meeting/2015

2016 Maize Meeting Website (Available November 2015)

http://maizegdb.org/maize_meeting/2016

Abstract Book (Electronic version)

http://maizegdb.org/maize_meeting/abstracts/2015Program.pdf

Cover Image (High-quality color)

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

The MaGNET Program and 2015 Awards

MaGNET (Maize Genetics Network Enhancement via Travel) is a program that seeks to recruit and retain scientists from diverse backgrounds into the maize research community by encouraging their attendance at the Annual Maize Genetics Conference (MGC). As such, it provides a source of support to help students and early career scientists from under-represented groups learn about maize genetics and connect with scientists already in the community. Awardees are not required to have previous maize genetics research experience, but will hopefully develop an appreciation of the current excitement in the field, and become an integral part of the community in the future. The program also provides an opportunity for awardees to explore potential collaborations and develop career contacts.

Each MaGNET Award helps defray the cost of attending the Maize Genetics Conference, including registration, food, lodging and airfare. In addition, awardees that have never attended the MGC are paired with an experienced 'Maize Mentor', who will help the awardee navigate the conference. Awardees are identifiable by a special notation on their name tags, and many of them are attending the MGC for the first time – please congratulate these scientists and welcome them to our famously hospitable conference!

All applicants must show strong potential for a career in the biological sciences, be either citizens or permanent residents of the USA, and belong to a group traditionally underrepresented in science. To help provide a more integrative and effective experience at the Conference for student awardees, faculty mentors who accompany one or more eligible student applicants are also eligible to apply for a MaGNET award.

2015 MaGNET Awardees

Undergraduate

Tylar Barnes, Norfolk State University	
Sean Colebrook, Oregon State University	Poster #307
Felix Fernandez-Penny, Cornell University	Poster #219
Jayla Harvey, Howard University	Poster #3
Sheena Vasquez, Georgia Perimeter College	Poster #4

Graduate Student

Dale Brunelle, University of North Dakota	Poster #249
Kayla Echols, Pennsylvania State University	Poster #190
Pablo Martinez, University of California, Riverside	Poster #250
Lorena Ríos-Acosta, University of Illinois, Urbana-Champaign	Poster #285

Scientist

Simara Price, University of Pennsylvania	Poster #365
Yolanda Serrano-Núñez, Inter American University, Bayamón Campus	

Faculty Mentor Accompanying Student

Camellia Okpodu, Norfolk State University	Poster #3

The MaGNET program of the Maize Genetics Conference is supported by grant IOS-1515023from the National Science Foundation.

Schedule of Events

Talks will be held in the St. Charles Ballroom.
Posters will be displayed in the Mega Center.

Thursday, March 12

2:30 PM – 6:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**
2:30 PM – 3:00 PM	**A Quick Tour of Araport: the Arabidopsis Information Portal** (Ruby Room)
3:00 PM – 4:00 PM	**"Gene Tagging with Green Ds" Maize workshop** (Ruby Room)
4:00 PM – 5:00 PM	**MaizeGDB Basics workshop** (Ruby Room)
5:00 PM – 6:00 PM	**MaizeGDB Advanced Usage workshop** (Ruby Room)
	Pre-registration recommended for the above sessions.
3:00 PM – 8:30 PM	**REGISTRATION** (Near Main Lobby)
3:00 PM – 6:00 PM	**POSTER HANGING** (Mega Center)
6:00 PM – 7:00 PM	**DINNER** (Mega Center)
7:00 PM – 9:00 PM	**SESSION 1 – PLENARY TALKS** Chair: Mark Settles Pages 23 & 24
7:00 PM	**WELCOME AND ANNOUNCEMENTS** (St. Charles Ballroom) Mark Settles
7:15 PM	**Paula McSteen, University of Missouri** [Plen 1] *Auxin and maize inflorescence development*
8:05 PM	**Xuemei Chen, University of California, Riverside** [Plen 2] *Small RNAs, a fascinating part of the transcriptome*
9:00 PM – 10:00 PM	**REGISTRATION** (Mega Center)
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY** (Mega Center)

Friday, March 13

7:00 AM – 8:00 AM	**BREAKFAST** (Mega Center)
7:00 AM – 8:15 AM	**REGISTRATION** (Mega Center)
8:00 AM – 10:15 AM	**SESSION 2 - BIOCHEMICAL GENETICS** Chair: Wes Bruce Talks 1-6. Pages 32-35
8:00 AM	**ANNOUNCEMENTS** (St. Charles Ballroom) Mark Settles
8:15 AM	**Vinzenz Handrick, Max Planck Institute for Chemical Ecology** [T1] *Elucidation of the final steps of benzoxazinoid formation in maize*
8:35 AM	**Vered Tzin, Boyce Thompson Institute for Plant Research** [T2] *Characterization of biological processes occurring in maize leaves in response to aphid feeding*
8:55 AM	**Shawn Christensen, USDA-ARS** [T3] *The maize death acids, 10-oxo-11-phytoenoic acid and derivatives, demonstrate specificity in jasmonate-related signaling and defense*
9:15 AM	**Guan-Feng Wang, North Carolina State University** [T4] *Molecular and Functional Analyses of a Maize Autoactive NB-LRR Protein Identify Precise Structural and Subcellular Requirements for Activity*
9:35 AM	**Junya Zhang, University of Florida** [T5] *The maize defective kernel5 (dek5) locus encodes a chloroplast-localized protein required for plastid division, membrane stability, and starch accumulation*
9:55 AM	**R. Frank Baker, University of Missouri** [T6] *Localization of maize SUCROSE TRANSPORTER1 reveals novel insights into phloem loading and sucrose retrieval*
10:15 AM	**BREAK**
10:45 AM – 12:25 PM	**SESSION 3 – MOLECULAR GENETICS** Chair: Petra Wolters Talks 7-11. Pages 36-38
10:45 AM	**Peng Yu, University of Bonn** [T7] *Local high nitrate modulates auxin transport in maize leading to altered lateral root initiation*
11:05 AM	**Neil Robbins, Stanford University** [T8] *Root hydropatterning: local water availability acts as a signal for lateral root initiation*
11:25 AM	**Rentao Song, Shanghai University** [T9] *Genome-wide characterization of cis-acting DNA targets reveals the transcriptional regulatory framework of Opaque2 in maize*
11:45 AM	**Erich Grotewold, Ohio State University** [T10] *Combinatorial Gene Regulation by R is Modulated by Small Molecule Interactions*
12:05 PM	**John Gray, University of Toledo** [T11] *The Maize TFome - development of a transcription factor open reading frame collection for functional genomics*

Friday, March 13 (continued)

12:30 PM – 1:30 PM **LUNCH** (Mega Center)

1:30 PM – 5:00 PM **POSTER SESSION 1** (Mega Center)

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.

4:40 PM – 6:00 PM **SESSION 4 – GENOME EDITING WORKSHOP**
Chair: David Jackson Workshop talks 1-4. Pages 27 - 30

4:40 PM **David Jackson, Cold Spring Harbor Laboratory**
Introduction to Genome Editing

4:45 PM **Stephen Novak, Dow AgroSciences** [W1]
A Modular and Flexible Gene Targeting System for Multigenerational Transgene Stacking in Plants

5:00 PM **Bing Yang, Iowa State University** [W2]
CRISPR/Cas9- and TALEN-mediated mutagenesis in rice and maize

5:15 PM **Jinjie Zhu, China Agricultural University** [W3]
Highly efficient and heritable targeted genome editing in maize using CRISPR-Cas9

5:30 PM **Mark Cigan, DuPont Pioneer** [W4]
CRISPR-Cas genome editing in maize

5:45 PM **Panel Discussion**

6:00 PM – 7:00 PM **DINNER** (Mega Center)

7:00 PM – 9:00 PM **SESSION 5 – MCCLINTOCK PRIZE PRESENTATION**
Chair: Carolyn Lawrence McClintock Talk. Page 31

7:00 PM **Carolyn Lawrence, Iowa State University**
McClintock Prize Presentation

7:30 PM **Sue Wessler, University of California at Riverside** [M1]
The Success Strategies of a Bursting Transposon

9:00 PM – 1:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY**
(Mega Center)

Saturday, March 14

7:00 AM – 8:00 AM **BREAKFAST** (Mega Center)

8:00 AM – 10:15 AM **SESSION 6 – CYTOGENETICS & GENOMICS**
Chair: Ruairidh Sawers Talks 12-17. Pages 39-42

8:00 AM **ANNOUNCEMENTS** (St. Charles Ballroom)
Mark Settles

8:15 AM **Wojtek Pawlowski, Cornell University** [T12]
Genomic features shaping the landscape of recombination hotspots in maize

8:35 AM **Fangpu Han, Chinese Academy of Sciences** [T13]
De novo centromere formation in maize

8:55 AM **Hank Bass, Florida State University** [T14]
Linking Chromatin Structure to Genomic Function through Differential Nuclease Sensitivity (DNS-seq) and Nucleosome Occupancy Mapping

9:15 AM **Candice Hirsch, University of Minnesota** [T15]
Insights into the relationship between structural diversity and transcriptional diversity in maize

9:35 AM **Gil Ronen, NRGene** [T16]
Comparing several maize reference genomes as a tool to discover conserved and variable key genetic elements in maize

9:55 AM **Robert Schaefer, University of Minnesota** [T17]
Integrating tissue specific co-expression networks with NAM GWAS to prioritize candidate gene sets

10:15 AM – 10:45 AM **BREAK**

10:45 AM – 12:25 PM **SESSION 7 – CELL & DEVELOPMENTAL BIOLOGY**
Chair: Amanda Wright Talks 18-22. Pages 43-45

10:45 AM **Byoung Il Je, Cold Spring Harbor Laboratory** [T18]
The maize FASCIATED EAR3 gene reveals a new CLAVATA signaling system, and controls ear size and stem cell proliferation by signaling from differentiating cells

11:05 AM **Samuel Leiboff, Cornell University** [T19]
Natural variation of maize shoot apical meristem morphology

11:25 AM **Sivanandan Chudalayandi, Iowa State University** [T20]
Cytokinin hypersignaling reprograms maize proximal-distal leaf patterning

11:45 AM **Katherine Petsch, Cold Spring Harbor Laboratory** [T21]
A novel DICER-LIKE1 pathway in maize buffers for loss of DICER-LIKE4 in tasiR-ARF biogenesis

12:05 PM **Michelle Facette, University of California, San Diego** [T22]
The SCAR/WAVE complex polarizes PAN receptors and promotes division asymmetry in maize

Saturday, March 14 (continued)

12:30 PM – 1:30 PM **LUNCH** (Mega Center)

1:30 PM – 5:00 PM **POSTER SESSION 2** (Mega Center)

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.

5:00 PM – 6:00 PM **COMMUNITY SESSION - Maize Genetics Executive Committee**
MGEC Chair: Carolyn Lawrence (St. Charles Ballroom)

6:00 PM – 7:00 PM **DINNER** (Mega Center)

7:00 PM – 8:55 PM **SESSION 8 – PLENARY TALKS**
Chair: Jinsheng Lai Pages 25 & 26

7:15 PM **Dirk Inzé, Ghent University, Belgium** [Plen 3]
Plant growth beyond limits

8:05 PM **Shawn Kaeppler, University of Wisconsin-Madison** [Plen 4]
Tuning developmental timing to maximize maize productivity

9:00 PM – 2:00 AM **INFORMAL POSTER VIEWING / DANCE** (Mega Center)

Sunday, March 15

7:00 AM – 8:20 AM **BREAKFAST** (Mega Center)

Posters should be taken down by 9 am!

8:20 AM – 9:50 AM **SESSION 9 – QUANTITATIVE GENETICS & BREEDING**
Chair: Sherry Flint-Garcia Talks 23-26. Pages 46-48

8:20 AM **ANNOUNCEMENTS** (St. Charles Ballroom)
Mark Settles

8:30 AM **Randy Wisser, University of Delaware** [T23]
Macroscopic microscopy: elucidating mechanisms of pathogen resistance for genes associated with quantitative defense

8:50 AM **Timothy Beissinger, University of California, Davis** [T24]
Patterns of demography and selection since maize domestication

9:10 AM **Xufeng Wang, China Agricultural University** [T25]
Dissecting the regulatory divergence between maize and its progenitor, teosinte

9:30 AM **Seth Murray, Texas A&M University** [T26]
Multi-parent and intermating population design effects on genetic mapping resolution of major color genes

9:50 AM **BREAK**

10:20 AM – 11:30 AM **SESSION 10 – TRANSPOSONS & EPIGENETICS**
Chair: David Braun Talks 27-29. Pages 49 & 50

10:20 AM **Elizabeth Buescher, Purdue University** [T27]
mop1 impacts the maternal contribution to maize seed

10:40 AM **Jonathan Gent, University of Georgia** [T28]
RNA-directed DNA methylation in suppression of intronic transposons: minimizing collateral damage on host gene expression?

11:00 AM **Erik Vollbrecht, Iowa State University** [T29]
Ds mutagenesis and a de novo W22 genome sequence

11:30 AM **ADJOURNMENT**

Posters

Computational and Large-Scale Biology

P1	**Christy Gault** <cg449@cornell.edu>	*A Preliminary Freezing Tolerance Screen in the Perennial Grass Genus Tripsacum, Which Is Closely Related to Maize*
P2	**Justin Walley** <jwalley@iastate.edu>	*An integrated system-wide maize atlas: from transcriptome to proteome networks*
P3	**Arun Durvasula** <adurvasula@ucdavis.edu>	*ANGSD-wrapper: scripts to streamline and visualize NGS population genetics analysis*
P4	**Chris Town** <cdtown@jcvi.org>	*Araport: the Arabidopsis Information Portal*
P5	**Fei Lu** <fl262@cornell.edu>	*Assembling maize inbred CML247: the maize pan-genome takes off*
P6	**Mingze He** <edifice1989@gmail.com>	*Assessing the prevalence and diversity of G-quadruplexes in regulatory regions of maize genes*
P7	**Shujun Ou** <oushujun@msu.edu>	*Automated construction of high-quality LTR exemplars from plant genomic sequences*
P8	**Jeff Glaubitz** <jcg233@cornell.edu>	*Biology of Rare Alleles in Maize and Its Wild Relatives*
P9	**Michael McKain** <mmckain@danforthcenter.org>	*Building a phylogenetic framework for comparative genomics in Andropogoneae*
P10	**Keting Chen** <kchen@iastate.edu>	*Computational exploration of the metabolic network of surface lipid production on maize silks*
P11	**Jianming Yu** <jmyu@iastate.edu>	*Consistent Non-Random Patterns in Nucleotide Base Composition across Genome-Wide Sequence Polymorphisms*
P12	**Charles Addo-Quaye** <caddoqua@purdue.edu>	*Detection of induced mutations in the resequenced genomes of 600 EMS-mutagenized Sorghum BTx623 individuals*
P13	**Xinxin Ding** <xding4@wisc.edu>	*Differential gene expression of maize aleurone and starchy endosperm cells at late developmental stages.*
P14	**Alina Ott** <alina.ott@gmail.com>	*Discovery and Mapping of Presence-Absence Variants (PAV) in the Founders of the Maize NAM Population*
P15	**Kelly Swarts** <kls283@cornell.edu>	*Dissecting evolutionary relationships of known genes with minimum spanning haplotype networks from maize HapMap3.1*
P16	**Xiang Li** <lixiang1989@webmail.hzau.edu.cn>	*Dissecting Meiotic Recombination based on Tetrad Analysis by Single Microspore Sequencing in Maize*
P17	**Karl Kremling** <kak268@cornell.edu>	*eQTL analysis to discover functional regulatory variation*
P18	**John Portwood** <john.portwood@ars.usda.gov>	*The Reinvention of MaizeGDB*
P19	**Ethalinda Cannon** <ekcannon@iastate.edu>	*Stewardship of the Maize B73 Reference Genome Assembly*
P20	**Miranda Leek** <archerygirl18@yahoo.com>	*MaizeGDB: Using GO for Gene Annotations*
P21	**Mary Schaeffer** <mary.schaeffer@ars.usda.gov>	*Diversity Panel Phenotype Data at MaizeGDB*
P22	**Jacqueline Richter** <richterj@iastate.edu>	*Comprehensive Comparison of MaizeCyc and CornCyc Metabolic Pathway Resources*

P23	**Paul Bilinski** <pbilinski@ucdavis.edu>	*Evolutionary genetics of repetitive DNA in maize and teosinte*
P24	**Wenbin Mei** <wmei@ufl.edu>	*Examining alternative splicing in maize and other grass genomes*
P25		(Poster withdrawn from abstract book)
P26	**Amanda Waters** <water157@umn.edu>	*Exploring allelic variation for response to abiotic stress*
P27	**Alexandra Asaro** <aasaro@wustl.edu>	*Gene by Environment Interaction in the Maize Ionome*
P28	**Wenwei Xiong** <xiongwenwei@gmail.com>	*Gene mutual information reveals regulatory network of maize embryo and endosperm development*
P29	**Shawn Thatcher** <Shawn.Thatcher@CGR.DuPont.com>	*Genome-wide analysis of alternative splicing in Zea mays: landscape and genetic regulation*
P30	**Marcela Monaco** <mmonaco@cshl.edu>	*Gramene: A resource for comparative plant genomics and pathways*
P31	**Indrajit Kumar** <ikumar@danforthcenter.org>	*High throughput TILLING-by-sequencing in grasses*
P32	**David Hufnagel** <davehuf@iastate.edu>	*Hybridization between highland and lowland teosinte populations in the Central Plateau and Balsas River Basin of Mexico*
P33	**Nicholas Haase** <nhaase@wisc.edu>	*Image-Based Precision Phenotyping of Maize Ear Morphology and Kernel Size*
P34	**Cory Hirsch** <hirsc213@umn.edu>	*Improving Our Biological Understanding of RNAseq*
P35	**Shuhua Zhan** <szhan@uoguelph.ca>	*Investigating the genetic basis of a maize gene coexpression network*
P36	**Ryan Douglas** <douglasrn@missouri.edu>	*Investigating the sequence, transcription, and translation of the maize B chromosome*
P37	**Ann Meyer** <ameyer@uoguelph.ca>	*Magnitude and causes of allelic differences in maize (Zea mays) short-read alignments*
P38	**Kokulapalan Wimalanathan** <kokul@iastate.edu>	*Maize - GO Annotation Methods Evaluation and Review (Maize-GAMER)*
P39	**Jessica Wedow** <wedow2@illinois.edu>	*Metabolite profiling of ozone stress response in Zea mays*
P40	**Kranthi Varala** <kv15@nyu.edu>	*Nutrinet : A Network inspired approach to improving Nutrient Use Efficiency (NUE) in crop plants.*
P41	**Jeff Gustin** <jgustin@ufl.edu>	*Phenotype discovery enabled by a machine vision pipeline for maize seed and seedling traits*
P42	**Christine Shyu** <CShyu@danforthcenter.org>	*Phenotypic analysis of stress responses in grasses using a high throughput phenotyping system and versatile analysis platform*
P43	**Benjamin Julius** <btjg2d@mail.missouri.edu>	*Phylogenetic analysis of SWEET transporters in angiosperms*
P44	**Simon Renny-Byfield** <sbyfield@ucdavis.edu>	*Presence-absence variants and estimates genome diversity in a population of wild maize*
P45	**Qing Li** <lixx3123@umn.edu>	*Regulation, natural variation and functional consequences of DNA methylation*
P46	**Lin Li** <lixx1601@umn.edu>	*Regulatory divergence of duplicate genes in maize*
P47	**Hao Wang** <wanghao@uga.edu>	*Repeat Junction Map Analysis of the B73 Maize Genome*

P48 **Garrett Janzen** <gjanzen@iastate.edu>
RNA-Seq and ecological niche analysis of a drought-resistant Mexican landrace

P49 **Scott Stelpflug** <stelpflug@wisc.edu>
The Expanded RNA-seq based Maize Gene Atlas: A focus on root development

P50 **Jacob Washburn** <jdwr47@mail.missouri.edu>
The Grass Tribe Paniceae and C_4 Photosynthetic Evolution

P51 **Ann Stapleton** <stapletona@uncw.edu>
The iPlant Collaborative: Cyberinfrastructure for enabling data to discovery

P52 **Yinping Jiao** <yjiao@cshl.edu>
The Maize Genome Project, an Update

P53 **Peng Liu** <mcliup@ufl.edu>
The path of assimilate delivery to developing maize kernels: Contrasting transcriptomes of the maternal phloem-unloading zone and the basal endosperm transfer cell layer

P54 **Samuel Seaver** <samseaver@gmail.com>
The PlantSEED resource for functional annotation and metabolic modeling of plant genomes, and the generation of tissue-specific metabolic models.

P55 **Arun Seetharam** <arnstrm@iastate.edu>
The teosinte (Zea mays ssp. parviglumis) de novo genome assembly and annotation

P56 **Sanzhen Liu** <liu3zhen@ksu.edu>
Understanding Maize Structural Variation Via BioNano Genome Mapping

P57 **Cliff Weil** <cweil@purdue.edu>
Utility to the Maize Community of a Functional Gene Discovery Platform for Sorghum Improvement

Biochemical and Molecular Genetics

P58 **Georg Jander** <gj32@cornell.edu>
5-Hydroxynorvaline, an abundant non-protein amino acid in maize

P59 **R. Frank Baker** <bakerrf@missouri.edu>
An update on bulk segregant analysis mapping of the carbohydrate partitioning defective mutants of maize

P60 **David Huizinga** <dhuizing@purdue.edu>
Using Bulk Segregant Analysis and Next-Generation Sequencing to Identify Novel Carbon Transport Genes in Maize

P61 **Kevin Chu** <chu16@purdue.edu>
A Biochemical Basis for Adult Plant Resistance in the Maize-CCR1 Pathosystem

P62 **Zhenyi Qiao** <qiaozhenyiinsh1987@163.com>
A maize MADS-box protein is identified to regulate the transcription of zein genes through its interaction with Opaque2

P63 **Weichang Yu** <wyu@cuhkri.org.cn>
A maize RWS-GFP haploid inducer line

P64 **Jonathan Saunders** <Jonosaun@ufl.edu>
A zebra-band phenotype results from mutation of a PPOX-like gene (protoporphyrinogen oxidase IX-like)

P65 **Weidong Wang** <wang4380@umn.edu>
Allele Specific Responses to Salt and UV in Maize

P66 **Mark Williams** <mark.e.williams@cgr.dupont.com>
Alternative Mutagens for Maize

P67 **Anna Rogers** <arrogers@iastate.edu>
Analysis of the maize cytokinin receptor Zea mays Histidine Kinase 1 function using Saccharomyces cerevisiae

P68 **Marcus McHale** <marcus.mchale@nuigalway.ie>
Assessing the potential for epigenetic gain in maize (Zea mays) hybrids through novel epiallele specific heterotic interactions

P69 **Tetyana Satarova** <satarova2008@yandex.ru>
Bioinformatical comparison of maize virus genomes

P70 **Maurice Paquette** <mpaquette2@mail.smcvt.edu>
Characterization and fine-mapping of maize carbohydrate partitioning defective13 mutant

P71	**Shangang Jia** <shangang.jia@gmail.com>	*Comparative Shotgun Proteomic Analysis of Isogenic Opaque Endosperm Maize Mutants*
P72	**Kevin Schneider** <kevinls@hawaii.edu>	*Dating Maize Centromere Divergence*
P73	**Robert Augustine** <raugustine@wisc.edu>	*Defining the SUMOylation System in Zea mays and its Roles in Stress Protection*
P74	**Sylvia M de Sousa** <sylvia.sousa@embrapa.br>	*Detailed expression analysis of maize Pstol1 homologs in contrasting genotypes for phosphorus efficiency*
P75	**Ross Zhan** <rzhan@purdue.edu>	*Determining Heat Tolerance via Chlorophyll Readings and Electrolyte Leakage*
P76	**Yinjie Qiu** <yinjie.qiu@sdstate.edu>	*Developing perennial maize for sustainable agriculture*
P77	**Hailey Karlovich** <hkarlovich01@hamline.edu>	*Developing protocols for understanding abiotic stress response in maize*
P78	**Maria Angelica Sanclemente** <sanangelma@ufl.edu>	*Dissecting putative roles of maize Pra1 and Ndpk1 in C-partitioning and energy balance*
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P268 **Christopher Kaiser** <kaiser8105@gmail.com>
Herbicide-safener GWAS for studying intraspecific variation in plant stress responses

P269 **Xin Li** <xinli@iastate.edu>
Heterosis of sorghum plant height caused by repulsion linkage in the Dw3 gene region

P270 **Jyoti Kaul** <kauljyoti1@yahoo.co.in> — *Identification of nutrient –dense germplasm for maize biofortification*

P271 **Nina Chumak** <nina.chumak@botinst.uzh.ch> — *Identification and Characterization of the Genetic Components of Apomixis in Maize (Zea mays L.)*

P272 **Rajdeep Khangura** <rkhangur@purdue.edu> — *Identification of a modifier of Oy1 (moy1) with the ability to boost photosynthesis*

P273 **Michaela Matthes** <micha.matthes@tum.de> — *Identification of candidate genes associated with carbon isotope discrimination and drought tolerance in maize*

P274 **Jack Gardiner** <jack.m.gardiner@gmail.com> — *Improving Standards and Methods for Phenotypic Prediction*

P275 **Tingting Guo** <tguo@iastate.edu> — *Insights from Classic Diallel Design into GWAS, GS, and Heterosis*

P276 **Xiaoqing Yu** <xyu@iastate.edu> — *Investigation of Several Critical Questions in Genomic Selection*

P277 **Alain Charcosset** <charcos@moulon.inra.fr> — *Joint Analysis of European Nested association Mapping Populations Reveals Different Multiallelic QTL for Hybrid Performance in the Flint and Dent Heterotic Groups of Maize*

P278 **Avinash Karn** <akarn@mail.missouri.edu> — *Joint-Linkage QTL analysis for total kernel protein content in teosinte near isogenic lines*

P279 **Rachel Paul** <paul9@illinois.edu> — *Leaf-level hyperspectral reflectance as a tool for measuring photosynthetic capacity in C4 grasses*

P280 **Peter Balint-Kurti** <pjbalint@ncsu.edu> — *Maize on the spot: Getting to the bottom of leaf lesions, flecking and spotting*

P281 **Moriah Massafaro** <mmassafa@purdue.edu> — *Mapping and identification of increased protein digestibility in sorghum*

P282 **Anna Krzywdzinski** <akrzywdz@uoguelph.ca> — *Mapping and identifying candidate genes of the modifier of amylose extender 1 (mae1) mutation in maize (Zea mays L.)*

P283 **Yijian He** <yhe9@ncsu.edu> — *Mapping of Quantitative Trait Loci for Salicylic Acid-induced Cell Death in intermated B73 x Mo17 (IBM) population*

P284 **Jiafa Chen** <JF.Chen@cgiar.org> — *Marker selection for fingerprint and quality control for CIMMYT maize lines*

P285 **Silvio Salvi** <silvio.salvi@unibo.it> — *Meta-QTL analysis for yield traits in maize*

P286 **Tes Posekany** <posekany@iastate.edu> — *Metabolite-QTL analysis of surface lipid production on maize silks: building statistical frameworks for inferences of biochemical function*

P287 **Ginnie Morrison** <morrisong@missouri.edu> — *Mild Inbreeding Depression in a Unique Synthetic Population: Preliminary Findings*

P288 **Darlene Sanchez** <darlenes@iastate.edu> — *Molecular characterization of doubled haploid exotic introgression lines in maize*

P289 **Aida Kebede** <Aida.Kebede@agr.gc.ca> — *Molecular mapping of gibberella ear rot resistance and kernel drydown rate in maize*

P290 **Fabian Strauss** <frs6493@louisiana.edu> — *Optimizing tissue culture parameters for callus induction and regeneration of transgenic sorghum Lines.*

P291 **Kate Crosby** <kcrosby@ucdavis.edu> — *Pedigree-based approaches to identifying selection in US maize*

P292 **Caroline Coatney** <ccoatney@uga.edu> — *Phenotypic Characterization of Traits Related to Perenniality in Maize/Teosinte*

P293 **Anna Selby** <acs5fd@mail.missouri.edu> — *Phenotypic evaluation of doubled haploids derived from the Zea Synthetic population*

P294 **Kokulapalan Wimalanathan** <kokul@iastate.edu>	*Pooled GBS: Cost-effective and background independent genetic mapping of mutants and QTL*
P295 **Samuel Bonfim Fernandes** <samuelfernandes@agronomo.eng.br>	*Predicting biomass yield in photoperiod-sensitive sorghum*
P296 **Yang Bian** <yang_bian@ncsu.edu>	*Prediction accuracy of QTL models improved by ensemble models*
P297 **Laura Morales** <lm596@cornell.edu>	*QTL mapping of resistance to Fusarium ear rot and fumonisin contamination in four NAM families*
P298 **Darshi Banan** <banan.darshi@gmail.com>	*Rapid Hemispherical Photographic Phenotyping of Productivity and Canopy Dynamics in a Setaria RIL Population*
P299 **Alain Charcosset** <charcos@moulon.inra.fr>	*Recovering Power in Association Mapping Panels with Variable Levels of Linkage Disequilibrium*
P300 **Seth Murray** <sethmurray@tamu.edu>	*Reducing pre-harvest losses from aflatoxin in maize production through integrated breeding and pest management strategies: initiation of a five year project*
P301 **Olga Danilevskaya** <olga.danilevskaya@pioneer.com>	*Reproductive Resilience of Drought Tolerant Hybrids under Water-Limited Conditions.*
P302 **Ashley Webster** <akschneider3@wisc.edu>	*Results of recurrent selection for large endosperm size in a supersweet population*
P303 **Masanori Yamasaki** <yamasakim@tiger.kobe-u.ac.jp>	*Rice Nested Association Mapping Population and its Phenotyping*
P304 **Vladyslav Cherchel** <vlad_cherch@mail.ru>	*Samples with high carotenoid and anthocyan contents in maize heterosis breeding*
P305 **Chutinan Jaroenchai** <cjaroenc@uoguelph.ca>	*Sink and Source Potential of Long-Ear Genetics*
P306 **Felix Seifert** <felix.seifert@uni-hamburg.de>	*sRNAome-based prediction of yield heterosis in maize*
P307 **Vivek Shrestha** <vivek.shrestha@sdstate.edu>	*Study of Quantitative Trait Polymorphisms Emerging From Doubled Haploids Maize Lines.*
P308 **Zhi Li** <lizhi0001@126.com>	*The Genetic Architecture of Maize Photoperiod Sensitivity Revealed by Multiple-parent Populations*
P309 **Denise Costich** <d.costich@cgiar.org>	*Uniting the world's popcorn diversity for the dissection of complex traits and accelerating breeding*
P310 **Jinliang Yang** <jolyang@ucdavis.edu>	*Utilizing Evolutionary Conservation Information to Improve Prediction Accuracy in Genomic Selection*
P311 **Gorka Erice** <erice@illinois.edu>	*Variation in yield loss to ozone of diverse inbred and hybrid maize lines*
P312 **Cinta Romay** <mcr72@cornell.edu>	*Yield and hybrid vigor within hybrids from inbreds preserved at the USA maize collection*
P313 **Greg Ziegler** <Greg.Ziegler@ARS.USDA.GOV>	*Zbrowse: An interactive GWAS results browser*

Transposons & Epigenetics

P314	**Yubin Li** <yubin@waksman.rutgers.edu>	*A sequence-indexed single gene knock-out resource for maize*
P315	**Weijia Su** <weijia@iastate.edu>	*Analysis of small RNA expression in maize normal and segmental duplication stocks*
P316	**Wei Xue** <wxue22@wisc.edu>	*Characterization of DNA methylation level in the Tourist transposable element and flanking region in the control region of maize domestication gene tb1*
P317	**Kaitlyn Socha** <sochak1@mail.montclair.edu>	*Characterization of full-length candidate genes capturing by Helitrons in B73*
P318	**Ji Huang** <jhuang@bio.fsu.edu>	*Characterizing and mapping of Transgene reactivated 9*
P319	**Jianhong Xu** <jhxu@zju.edu.cn>	*Differential DNA methylation of 19-kDa zein genes in maize*
P320	**Kameron Wittmeyer** <ktw5072@psu.edu>	*Distinct mechanisms of chromatin related silencing pathways of the ufo1 and Mop1 genes in maize.*
P321	**Ryan Douglas** <douglasrn@missouri.edu>	*Examining the transcriptional changes involved in maize centromere inactivation and reactivation*
P322	**Cristian Forestan** <cristian.forestan@unipd.it>	*Genetic and epigenetic regulation of maize transcriptome and genome stability under stress conditions: from chromatin modification to lncRNAs and beyond*
P323	**Bosen Zhang** <bszhang@illinois.edu>	*Genetic variation for retrotransposon derived small RNAs in maize*
P324	**Qixian Tan** <qzt101@psu.edu>	*Identification and transcriptome analysis of a silent allele of Unstable factor for orange 1*
P325	**Eli Rodgers-Melnick** <er432@cornell.edu>	*Open Chromatin Reveals the Functional Portion of the Maize Genome*
P326	**Charles Hunter** <cthunter3@gmail.com>	*Over 9,000 new mutants added to UniformMu: 66,000 total Mu insertions with 40% genome coverage*
P327	**Stefan Scholten** <s.scholten@uni-hohenheim.de>	*Pericentromeric 22-nt small RNAs suppress heterosis in maize*
P328	**Nicholas Heller** <njhelle2@illinois.edu>	*Production and Characterization of a Population of Epigenetic NILs*
P329	**Susanne Edelmann** <susanne.edelmann@uni-hamburg.de>	*Reduction of DNA methylation during early embryogenesis enhances growth heterosis of maize plants*
P330	**Joy-El Talbot** <talbot.52@osu.edu>	*Tandem repeats are implicated in both the establishment and maintenance of paramutation at Pl1-Rhoades*
P331	**Xiaomei Dong** <wawjdxm@163.com>	*The dynamic changes of genetic imprinting in the progress of maize endosperm development*
P332	**Tao Zuo** <taozuo@iastate.edu>	*The mechanism and impact of alternative transposition-induced DNA re-replication in maize*
P333	**Linda Stroud** <lstroud@bio.fsu.edu>	*The role of RdDM chromatin proteins in nucleosome occupancy.*
P334	**Michelle Stitzer** <mcstitzer@ucdavis.edu>	*Transposable Element Polymorphism Impacts Gene Expression in Maize Inbred Lines*
P335	**Irina Makarevitch** <imakarevitch01@hamline.edu>	*Transposable elements contribute to activation of maize genes in response to abiotic stress*
P336	**Dongyan Zhao** <zhaodon4@msu.edu>	*Transposition of a Rice Mutator-Like Element in the Yeast Saccharomyces cerevisiae*
P337	**Quan Zhang** <qzhang@danforthcenter.org>	*Using Ac/Ds transposon mutagenesis to characterize the function of maize genes involved in mycorrhizal signaling pathway*

Genome Editing

P338 **Scott Zarecor** <szarecor@iastate.edu> — *CTGA: The CRISPR TALEN genome analyzer*

P339 **Sarah Briggs** <sabriggs@iastate.edu> — *Heritable site-specific gene mutagenesis using TALENs in maize*

Late Poster Abstracts

P340 **Avimanyou K. Vatsa** < akvhxd@mail.missouri.edu> — *Characterization of Low-Dimensional Complex Phenotypes*

P341 **Toni Kazic** <kazict@missouri.edu> — *What Can We Learn From Complex Phenotypes?*

Plenary Talk Abstracts

Plenary 1 **Thursday, March 12 7:15PM**

Meristems, mutants and maize: genetic insights into fundamental processes in reproductive development

(presenter: Paula McSteen <mcsteenp@missouri.edu>)
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Axillary meristems (groups of stem cells that arise in the axils of leaves) play a fundamental role in plant architecture and reproduction. In maize, axillary meristems produce the tillers, ear shoots, and long and short branches (called spikelets) in the tassel and ear. My lab uses a genetic approach to identify genes required for axillary meristem development by isolating mutants, including the *barren inflorescence* (*bif*) class of mutants, which fail to initiate branches and spikelets in the tassel and kernels in the ear. These screens have led to the identification of gene families required for the biosynthesis, transport and perception of the plant growth hormone auxin. Another class of mutants that fail to make an ear, called *barren stalk* (*ba*) mutants, has led to the identification of transcription factors acting downstream of auxin in axillary meristem development. A third class of mutants that fail to make a tassel, called *tassel-less* (*tls*) mutants, have revealed new roles for nutrients in meristem maintenance. Collectively, these studies have revealed differential hormonal and genetic regulation of apical and axillary meristems. All of these mutants affect both the tassel and ear, illustrating their shared developmental programs. This talk will highlight our current knowledge of tassel and ear formation which is essential for understanding the developmental processes involved in maximizing maize yield.

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

Mechanisms of microRNA turnover in Arabidopsis

(presenter: Xuemei Chen <xuemei.chen@ucr.edu>)
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microRNAs (miRNAs) are a class of small regulatory RNAs with large impacts in almost all biological processes in plants and animals. It is thus important to understand the molecular mechanisms underlying the biogenesis and degradation of miRNAs. While the framework of miRNA biogenesis has largely been elucidated, the mechanisms that underlie the turnover of mature miRNAs are not well understood in either plants or animals. We have identified several families of enzymes, including exonucleases and nucleotidyl transferases, that turnover miRNAs in Arabidopsis. The exonucleases truncate miRNAs from the 3' end and the nucleotidyl transferases add a short, U-rich tail to miRNAs. Two nucleotidyl transferases, HESO1 and URT1, show different substrate preferences and act coordinately in vivo to tail miRNAs. Both enzymes can act on ARGONAUTE1 (AGO1)-bound miRNAs, and tailing reduces the slicing activity of miRNAs in addition to causing miRNA degradation. I will discuss these and other mechanisms of miRNA degradation.

Plant growth beyond limits

(presenter: Dirk Inze <Dirk.inze@psb.vib-ugent.be>)
Full Author List: Inze, Dirk[1]; Muszynski, Michael[2]; Cabral, Luiz[1]; Sun, Xiaohuan[1]; Van Lysebettens, Mieke[1]; Nelissen, Hilde[1]
[1] Department of Plant Systems Biology, VIB-UGent, Belgium
[2] Genetics, Development, and Cell Biology, Iowa State University, USA

Plant and plant organ growth are regulated by an exceedingly complex interplay of many genes and their interaction with the ever changing environment. Maize leaf development offers great opportunities to study the dynamics of growth regulatory networks, essentially because leaf growth can be viewed as a linear system in which three zones form a gradient along the length of the leaf: the division zone (DZ) in which cells proliferate is positioned at the base of the leaf, followed by the elongation zone (EZ) in which cells expand, and the mature zone (MZ). These zones are separated by transition zones, termed TZ1 and TZ2, between division and expansion and expansion and mature, respectively. The importance of the position of TZ1 for final leaf size became clear when growth analysis revealed that in plants overexpressing *GA20OX1*, the rate-limiting enzyme of GA biosynthesis, the leaf was 40% longer due to a 40% larger DZ. The significance of TZ1 in determining final leaf size is also reflected in cellular analysis of leaves exposed to mild drought conditions, that reduces the growth of the fourth leaf by 30%, without causing wilting or leaf rolling. An additional advantage of the maize leaf is its size that allows for a high spatial resolution sampling throughout the growth zone. To this end, we performed a comprehensive analysis of gene expression with extensive temporal and cell-type specific resolution that provided novel insights on the growth regulatory processes that accompany cell proliferation and its transition to cell expansion.

Numerous genes of which the modified expression enhances plant organ growth have now been identified, and a detailed study of these genes and combinations between them triggered the compilation of individual building blocks into molecular networks driving growth. Indeed, evidence in the Arabidopsis demonstrated that the combination of multiple growth enhancing genes can have very profound effects on organ size. Similarly, in maize some combinations were found to have an additive effect organ growth, again demonstrating that gene combinations are the solution to significantly enhance organ growth in plants. Field trials showed that some of these genes not only enhance leaf growth, but also positively affect ear growth and floret number. Our research opens up new perspectives for the identification of optimal growth regulatory networks that can be selected for by advanced breeding, or for which more robust variants (e.g. reduced susceptibility to drought) can be obtained through genetic engineering.

Tuning developmental timing to maximize maize productivity

(presenter: Shawn Kaeppler <smkaeppl@wisc.edu>)
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The massive grain yield achieved in 2014 is not due to events of a single year, but results from a continuum of integrated and critical activities. Breeders made the initial crosses for hybrids grown in 2014 five to ten or more years ago. Selection across generations and locations identified commercial hybrids and seed was amplified for sale in the 2014 season. Farmers began ordering seed in mid-2013, and the logistical process to deliver the nutrient and chemical inputs to support the crop was underway. The legacy of this crop will persist not only through the food, feed, and raw materials produced, but also in components such as the agricultural residues that remain and will contribute to upcoming seasons. In the context of this timeline to product, I will highlight some of the major biological components of the maize developmental program. I will discuss some of our research utilizing natural variation and genomics to dissect components of this developmental program. Finally, I will consider how components of the developmental program may be adapted to facilitate new production systems including components such as cover crops. As researchers, the decisions and discoveries that we make today will contribute to the crop ten or more years in the future. Therefore, anticipating and understanding trends in the timelines that coalesce to produce the amazing and necessary yields for the future is critical to maximize the potential of maize.

Funding acknowledgement: Department of Energy (DOE), National Science Foundation (NSF), USDA-AFRI, USDA-Hatch

Genome Editing Workshop Abstracts

Workshop 1 **Friday, March 13 4:45PM**

A Modular and Flexible Gene Targeting System for Multigenerational Transgene Stacking in Plants

(presenter: Stephen Novak <snnovak@dow.com>)
Full Author List: Kumar, Sandeep[1]; AlAbed, Dia[1]; Worden, Andrew[1]; Bennett, Sara[1]; Gupta, Manju[1]; Wu, Huixia[1]; Ausmus, Carla[1]; Beck, Margaret[1]; Robinson, Heather[1]; Hemingway, Daren[1]; Foulk, Stephen[1]; Chen, Wei[1]; Skaggs, Nicole[1]; Lutz, Jamie[1]; Novak, Stephen[1]
[1] Dow AgroSciences LLC, Indianapolis, IN, 46268

A selection-based multigenerational and modular method for precise integration of transgenes into plant genomes has been developed for creating a multi-gene stack using zinc finger nuclease (ZFN)-mediated double strand breaks in the pre-defined target genomic location. This precision targeting strategy utilizes a unique intron present at the 3' end of a promoter driving a selectable marker gene to facilitate homology between target and donor molecules such that only insertion into the target locus leads to a functional selectable marker. The random insertions of the promoter-less donor molecule are eliminated on a positive selection media leading to high-frequency gene targeting. The new stack of transgenes is loaded with each generation of gene targeting swapping the selectable marker gene using the intron homology. This system was tested in maize using the PAT selectable marker gene and up to 30% targeting among plants regenerated on selection medium was observed. Unlike previous gene targeting methods that utilize defective or partial genes to select for targeted events, the present method swaps fully functional genes with every cycle of targeting. This unique feature makes the targeting system completely modular and flexible and thereby could be extended to multiple generations, selectable markers and/or ZFN recognition sites with every round of gene targeting and stacking. The gene targeting strategy described here in maize is believed to be of general applicability across all crop species where transformation is possible.

Funding acknowledgement: Dow AgroSciences LLC

CRISPR/Cas9- and TALEN-mediated mutagenesis in rice and maize

(presenter: Bing Yang <byang@iastate.edu>)
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Biotechnologies derived from engineered nucleases such as modified Cas9/sgRNA (Clustered Regularly Interspaced Palindromic Repeat single guide RNA/CRISPR associated protein 9) and TALENs (transcription activator-like effector nucleases) have been recently developed and widely utilized for targeted gene mutagenesis in many organisms, including agriculturally important plants such as rice, wheat, barley and maize. My presentation describes development and application of these two technologies to generate heritable genome modifications in rice and maize. TALENs were employed to generate stable, heritable mutations in dozens of rice genes and maize glossy2 locus. The frequency of mutagenesis varied from species and constructs targeting different genes. The highest rate in rice reached about 65% of transgenic lines carry desired mutations, while construct targeting the maize glossy2 gene produced mutant lines from the maize genotype Hi-II at a frequency of about 10% (9 mutated events in 91 transgenic events). Phenotypes associated with mutated genes were observed in some mutant lines in rice and maize. We also modified the Cas9/sgRNA system suitable for targeted gene mutagenesis in rice and maize, respectively. The two systems have been successfully applied to rice and maize genes for targeted mutagenesis. Transgenic lines of T0 generation carrying site-specific mutations were produced at frequency as high as 100% in rice and 80% in maize. The on-going characterization of the inheritability of some mutations in maize will also be presented. Our results demonstrate that TALENs and Cas9/sgRNA are effective toolboxes for genome mutagenesis in rice and maize, empowering the discovery of gene function and the development of trait improvement.

Funding acknowledgement: The Iowa State University Crop Bioengineering Consortium

Highly efficient and heritable targeted genome editing in maize using CRISPR-Cas9

(presenter: Jinjie Zhu <zhujinjie820@126.com>)
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Targeted genome editing, the ability to manipulate a genome precisely, site-specifically and permanently, has been an important goal of plant biotechnology for decades. Recently, an adaptive immunity system from bacteria and archaea to defend against foreign DNA fragments repeated invasions has become a straightforward genome editing tool for Eukaryotic cells. The site-specificity of the system is governed by a small non-coding RNA, which has significant advantages compared with alternative DNA binding protein systems. Here we configured the well-studied type Ⅱ CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR- associated proteins) for targeted genome modification in maize and characterized a functional maize U6 snRNA promoter to express the short non-coding targeting RNA. We established a database for CRISPR-Cas9 targets in exonic regions with minimally off-target loci matches, covering more than 95% of the current maize filtered gene set. We detected successful specific cleavage activity for 76 locus with a transient protoplast assay, average cleavage efficiency is about 11.89%. Then we knocked out the endogenous maize Y1 gene in T0 transformed plants. The expected albino phenotype in recessive mutant of Y1 with two different mutated alleles, and mosaic leaf phenotype in chimeric transgenic plants were observed. The mutation penetration efficiency in seedling of stable transgenic plants ranged from 65%-100%. Mutation types include deletions, insertions and deletions accompanied by insertions, predominantly 1bp insertion and short deletions (<10bp). Mutations occurred in germ cells can be highly inheritated to next generation. The successful application of the CRISPR-Cas9 system in Zea mays demonstrates this facile, robust, and wide applicable system can be employed for functional genetics and crop improvement research.

Funding acknowledgement: National Natural Science Foundation of China (grant no.31225020; 31421005; 91435206) and National High Technology Research and Development of China (863 Project, grant no.2012AA10A305) and the 948 project (2011-G15).

CRISPR-Cas genome editing in maize

(presenter: Mark Cigan <mark.cigan@pioneer.com>)
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Historically, plant breeders have relied on extant variation and genetic selection in adapted germplasm to develop, study and improve traits critical for increased yield and productivity in agronomically important food and feed crops. This process has been accelerated in part by the sequencing of plant genomes and the introduction of plant transformation methods to insert genes or traits. The ability to induce genetic variation, advance plant gene studies, and develop unique traits by simple, inexpensive and precise methods to direct the modification or insertion of genes and traits has been viewed as the next breakthrough necessary for agriculture to meet the needs of a growing population. The rate-limiting step in genome editing has been the ability to produce and direct double-strand-breaks (DSBs); however with the introduction of protein-based DSB reagents like zinc-finger nucleases, homing endonucleases (meganucleases) and TALENs, plant biotechnology began to take steps towards realizing a revolutionary change in agriculture. Most recently, a DSB system of bacterial origin known as CRISPR (Clustered Regularly Interspersed Short Palindromic Repeats) has been discovered in which protein-RNA complexes (Cas9-guideRNA) provide natural immunity against invading foreign DNA. Using *Streptococcus* derived components, a programmable RNA-guided DNA endonuclease system has been developed providing a simple, inexpensive and universal platform for genome editing. Not surprisingly, Cas9-guideRNA has already been demonstrated as a genome editing tool in many eukaryotic systems including human, mouse, zebrafish, *Drosophila*, *C. elegans*, yeast and plants. The Genome Modification Group at DuPont Pioneer uses a variety of DSB approaches in its research programs to modify the corn, rice, sorghum and soybean genomes for the purpose of gene editing and gene stacking. Examples of Cas9-guideRNA directed genome modification will be presented to demonstrate the power this new system offers to study plant genetics and biology.

McClintock Prize Abstract

McClintock Prize **Friday, March 13 7:30PM**

The Success Strategies of a Bursting Transposon

(presenter: Sue Wessler <susan.wessler@ucr.edu>)
Full Author List: Wessler, Sue[1]; Stajich, Jason[1]; Okumoto, Yutaka[2]; Lu, Lu[1]; Chen, Jinfeng[1]; Robb, Sofia[1]; Shi, Jinghua[1]
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Transposable elements (TEs) comprise the largest proportion of all characterized plant and animal genomes. In part, this reflects the ability of a few TEs in a genome to undergo a "burst" – a term that describes the rapid increase in number to thousands, even tens of thousands of copies. In plants, two TE types are associated with bursts: class 1 LTR retrotransposons and class 2 miniature inverted repeat transposable elements (MITEs). While the former have a tendency to insert in intergenic regions, usually into other LTR retrotransposons, MITEs attain copy numbers of hundreds and thousands despite a preference for genic regions. How MITEs do this without killing their host or being silenced is the focus of our studies.

Although MITEs were first discovered in maize, rice (*Oryza sativa*) has proven to be our organism of choice for determining their strategies for success. First, the small genome of rice facilitated the computer-assisted discovery of *mPing*, the first actively transposing MITE and Ping, the autonomous element responsible for its movement. A strategy for *mPing*'s ability to rapidly increase in copy number was revealed to be a preference for insertion into noncoding genic regions and an avoidance of exons. Second, as a predominantly self-pollinating organism, pure lines of rice can be propagated for decades. We have exploited this feature to determine the genome-wide impact of *mPing* bursts in two pairs of rice strains, each derived recently from a common ancestor: EG4/HEG4 and A123/A119. Comparative analysis of their sequences provide evidence that (1) the strain pairs have been maintained as pure lines for ~20 and ~100 years, respectively, (2) the bursts have been sustained for decades in the presence of what appears to be normal genome surveillance, and (3) the bursts were probably not initiated by what McClintock called "genome shock".

Short Talk Abstracts

SESSION 2 - BIOCHEMICAL GENETICS
Chair: Wes Bruce Friday, March 13. 8:15 AM – 10:15 AM

T1

Elucidation of the final steps of benzoxazinoid formation in maize

(presenter: Vinzenz Handrick <vhandrick@ice.mpg.de>)
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[3] Boyce Thompson Institute for Plant Research, Ithaca, NY 14853, USA

Benzoxazinoids are plant defense compounds which are mainly produced in graminaceous plants. They have been shown to act against major pests of cultivated maize such as fungi (e.g. Northern corn leaf blight) and insects (e.g. European corn rootworm and maize leaf aphid). The biosynthesis of benzoxazinoids in maize has been extensively studied. The core pathway starts with the conversion of indole-3-glycerol phosphate to indole and includes subsequent oxidations, methylations and a glucosylation reaction leading to the formation of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside (DIMBOA-Glc). After insect herbivory, DIMBOA-Glc **(1)** can be further converted to other benzoxazinoids such as DIM_2BOA-Glc **(2)** and HDM_2BOA-Glc **(3)** which has been assumed to increase resistance to herbivores. However, the biosynthetic sequences leading to **2** and **3** as well as the involved enzymes are currently not known.

Quantitative trait locus mapping revealed that the formation of **2** is associated with a 2-oxoglutarate dependent dioxygenase gene, *ZmBx11*, and an *O*-methyltransferase gene, *ZmBx12*. Heterologous overexpression of *ZmBx11* in *Escherichia coli* and subsequent enzyme assays with purified recombinant protein showed that recombinant ZmBX11 was able to hydroxylate **1** at position 8, forming the intermediate TRIMBOA-Glc **(4)**. Similarly, heterologously expressed ZmBX12 produced **2** by the methylation of **4**. Furthermore, we identified another *O*-methyltransferase, ZmBX13, which was able to convert **2** into **3**. The transcripts of *ZmBx11*, *ZmBx12*, and *ZmBx13* were found to be induced concertedly upon herbivory in maize leaves and roots which correlated with a higher accumulation of **2** and **3** in these tissues in comparison to undamaged plants. A maize line with an inactive ZmBx11 allele showed no accumulation of **2** and **3** and choice assays demonstrated that this mutant line was more attractive to the root feeding herbivore *Diabrotica virgifera* than a near isogenic line possessing an active *ZmBx11* allele. This suggests a possible role for **2** and **3** in plant defense against insect herbivores.

Funding acknowledgement: Swiss National Science Foundation (SNSF)

T2

Characterization of biological processes occurring in maize leaves in response to aphid feeding

(presenter: Vered Tzin <vt223@cornell.edu>)
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More than 90 herbivorous insect species are known to attack maize (Zea mays), resulting in losses ranging from 6% to 19% of total productivity. Aphids and other hemipteran pests cause direct damage to maize by sucking phloem nutrients as well as indirect damage by transmission of plant viruses. To survive insect attack, maize plants have evolved constitutive and inducible chemical and physical defenses. We aimed to elucidate the metabolic processes that are induced in maize leaves during aphid attack. Leaves of inbred line B73 were infested with corn leaf aphids (Rhopalosiphum maidis) and leaf samples were collected at time points ranging from 0 to 96 hours. Gene expression profiling by next-generation sequencing, HPLC, HPLC-MS, and GC-MS were used to generate large transcriptomic and metabolomic datasets. Overall, these analyses showed that aphid feeding on maize leaves causes a metabolic shift from growth to the production of chemical defenses. Major transcriptional changes included the up-regulation of biosynthetic pathways for aromatic amino acids, methionine, and salicylic acid. The phytohormone jasmonic acid, which is known to be produced in response to insect herbivory, was highly induced after 24 hours, and then dropped back down to the basal level. Additionally, for several genes that were greatly induced in response to aphid feeding, including genes encoding the benzoxazinoids pathway, biosynthesis of jasmonic acids, terpenoid synthesis and receptor-like protein kinase, we identified Ds transposon insertion knockout lines to characterize the function of these genes in maize response to aphid attack.

Funding acknowledgement: Vaadia-BARD Postdoctoral Fellowship award

T3

The maize death acids, 10-oxo-11-phytoenoic acid and derivatives, demonstrate specificity in jasmonate-related signaling and defense

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Plant cellular damage promotes the interaction of lipoxygenases (LOX) with free fatty acids to yield 9- and 13-hydroperoxides which are further metabolized into diverse oxylipins. The enzymatic action of 13-LOX on linolenic acid enables production of 12-oxo-phytodienoic acid (12-OPDA) and its downstream products, jointly known as jasmonates. As signals, jasmonates have related yet distinct roles in the regulation of plant resistance against insect and pathogen attack. An additional and conceptually parallel pathway involving 9-LOX activity on linoleic acid leads to the production of 10-oxo-11-phytoenoic acid (10-OPEA). Despite structural similarity to jasmonates, physiological roles for 10-OPEA have remained unclear. In developing maize (Zeamays) leaves, fungal infection by Southern leaf blight (Cochliobolus heterostrophus) results in the localized production of 10-OPEA and a series of related 12- and 14-carbon metabolites, collectively termed ‘death acids’. While typically absent, 10-OPEA becomes highly wound-inducible within fungal-infected tissues. As a direct defense, 10-OPEA suppresses the growth of mycotoxigenic fungi, including Aspergillus flavus and Fusarium verticillioides, and also the insect herbivore Helicoverpa zea. Both 12-OPDA and 10-OPEA equally promote the transcription of numerous defense genes encoding glutathione S-transferases, cytochrome P450s, and pathogenesis-related proteins; however, 10-OPEA activity diverges in the context of reduced protease inhibitor transcript accumulation. Consistent with a role in dying tissue, 10-OPEA exhibits significant potency and specificity in triggering ion leakage and cell death, which is significantly impaired by the cysteine protease inhibitor maize cystatin-9. Unlike widely encountered jasmonates, functions of 10-OPEA and associated death acids are consistent with specialized roles in local defense reactions.

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

T4

Molecular and Functional Analyses of a Maize Autoactive NB-LRR Protein Identify Precise Structural and Subcellular Requirements for Activity

(presenter: Guan-Feng Wang <gwang11@ncsu.edu>)
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Plant disease resistance is often mediated by nucleotide binding-leucine rich repeat (NLR) proteins that trigger a rapid localized programmed cell death termed the hypersensitive response (HR) upon pathogen recognition. Three domains are recognized in one of the major classes of NLR proteins: a coiled-coil (CC), a nucleotide binding (NB-ARC) and a leucine rich repeat (LRR) domains. The maize NLR gene *Rp1-D21* derives from an intergenic recombination event between two NLR genes, *Rp1-D* and *Rp1-dp2* and confers an autoactive HR. We report systematic structural and functional analyses of Rp1 proteins in maize and *N. benthamiana* to characterize the molecular mechanism of NLR activation/auto-inhibition. We derive a model comprising the following three main features: Rp1 proteins appear to self-associate to become competent for activity. The CC domain is signaling-competent and is sufficient to induce HR. This can be suppressed by the NB-ARC domain through direct interaction. In autoactive proteins, the interaction of the LRR domain with the NB-ARC domain causes de-repression and thus disrupts the inhibition of HR.

Rp1-D21 was predominantly localized in cytoplasm with a small amount in nucleus. Targeting of Rp1-D21 to either nucleus or cytoplasm abolished HR-inducing activity, suggesting nucleocytoplasmic movement was important for HR induction.

From GWAS analysis in maize, we identified a gene (*Lig*) predicted to be involved in the lignin biosynthesis pathway, which is associated with natural variation in the severity Rp1-D21-mediated HR phenotype. Lig suppresses Rp1-D21-induced HR in *N. benthamiana* when transiently co-expressed with Rp1-D21. Furthermore Lig physically associates with Rp1 proteins in co-immunoprecipitation and bi-molecular fluorescence complementation assays.

In summary, this work reports several novel insights into the precise structural and subcellular requirements for NLR function and identifies a novel co-factor, Lig, which regulates Rp1-D21 autoactivity through direct interaction.

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

T5

The maize *defective kernel5* (*dek5*) locus encodes a chloroplast-localized protein required for plastid division, membrane stability, and starch accumulation

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The *defective kernel 5* (*dek5*) locus conditions a severely reduced starchy endosperm with a shrunken phenotype similar to starch biosynthetic mutants such as *brittle1* and *brittle2*. Typical endosperm starch biosynthetic mutants do not affect seedling or plant development. By contrast, *dek5* mutants disrupt embryo development or produce an 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 chloroplasts when compared to normal siblings. These enlarged chloroplasts show a range defective to completely normal internal membrane ultrastructure. Particle size analysis of mid-development starch granules revealed that *dek5* has enlarged starch granules, suggesting *dek5* endosperm amyloplasts are also enlarged relative to normal sibling kernels. By co-localizing genetic mapping position with *Mu*-Seq data from *dek5-25* , we identified the *dek5* locus. . Complementation tests with alleles from UniformMu reverse genetics resources as well as sequencing additional *dek5* alleles from the Maize Genetics Cooperative Stock Center confirmed the identity of the *dek5* gene. *dek5* encodes a 2,123 amino acid protein from a 30 kbp gene. Subcellular localization experiments show DEK5 is a chloroplast-localized protein. 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* has a role in plastid division and internal plastid membrane stability. These data suggest plastid division is critical for amyloplast function of the starchy endosperm as well as in the chloroplasts of seedling leaves.

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

T6

Localization of maize SUCROSE TRANSPORTER1 reveals novel insights into phloem loading and sucrose retrieval

(presenter: R. Frank Baker <bakerrf@missouri.edu>)
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Plant growth, development, and ultimately crop yield are dependent on the transport of photosynthates from the leaves to non-photosynthetic sink tissues (e.g., ears, tassels, roots). For long-distance transport, sucrose is loaded into the phloem in leaf minor veins by sucrose transporters (SUTs). In some plants, the phloem companion cells (CCs) are the sites for sucrose loading, while in other plants the phloem sieve elements are proposed to be the cells responsible for sucrose uptake. In maize, it remains unknown which cells are involved in sucrose entry into the phloem. To address this question, we characterized the expression of the maize *Sucrose transporter1 (ZmSut1)* gene, which was previously proposed to function in sucrose phloem loading based on its expression and mutant phenotype. For elucidating the expression pattern of the *ZmSut1* transcript, we performed RNA *in situ* hybridizations on mature leaf tissues. These experiments revealed *ZmSut1* was expressed in CCs, supporting a role for this gene in phloem loading. Surprisingly, we also detected strong expression of *ZmSut1* in non-conducting cells in leaf veins, suggesting *ZmSut1* also functions in sucrose retrieval from the apoplasm in cells located peripherally to the conducting cells. Analyses of a *ZmSut1* promoter: RFP transcriptional reporter transgene confirmed and extended these findings, and indicated *ZmSut1* expression occurs in the veins of both mature and immature tissues throughout the plant. To determine the subcellular localization of the ZmSUT1 protein, a translational reporter fusion to YFP under the control of the native regulatory sequences was examined. These studies demonstrated ZmSUT1 localizes to the plasma membrane. Collectively, these findings indicate that ZmSUT1 functions to actively load sucrose into the phloem in the CCs. Additionally, they provide evidence that *ZmSut1* functions to recover sucrose lost to the apoplasm far from the conducting cells, and that this sucrose recycling is important for maize growth.

Funding acknowledgement: National Science Foundation (NSF)

SESSION 3 - MOLECULAR GENETICS
Chair: Petra Wolters Friday, March 13. 10:45 AM – 12:25 PM

T7

Local high nitrate modulates auxin transport in maize leading to altered lateral root initiation

(presenter: Peng Yu <yupeng@uni-bonn.de>)
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The phenotypic plasticity of lateral roots which are initiated from specific pericycle cells upon external stimulation allows them to compete for nutrients in patchy microenvironments. The present study aimed to decipher post-embryonic lateral root initiation mechanisms in maize and their response to heterogeneous nitrate.

Morphological and histological analyses demonstrated that cell division activity peaked at a distance between 5 and 25 mm from the root tip in response to local high nitrate compared to homogeneous low nitrate as a control. Comparative transcriptome profiling of manually dissected steles of nitrate-treated brace roots revealed differentially expressed genes involved in auxin-induced cell division or cell fate processes. To explore the role of auxin in maize lateral root initiation, the auxin response reporter DR5::RFP was employed to survey the active role of auxin on priming pericycle cells during lateral root initiation. By using a modified lateral root inducible system, we observed that the auxin maxima in phloem pole cells can be restored by local high nitrate stimuli, while low nitrate failed to relieve the inhibition caused by the auxin transport inhibitor NPA. Tissue-specific measurements of active IAA determined by UPLC-ESI-MS/MS revealed auxin redistributed from root tip and outer cortex to the stele navigated by dynamic expression of tissue-specific ZmPIN1. Based on RNAseq results, the monocot-specific PIN-FORMED (PIN) auxin efflux transporter ZmPIN9 was upregulated by local high nitrate. Moreover, linear induction of ZmPIN9 was observed in the stele by qRT-PCR. The novel N-induced auxin transporters were differentially expressed in diverse cell types including phloem poles and their pericycle and endodermis cells related to lateral root initiation. These cells were previously separated by laser capture microdissection. Taken together, these results highlight a novel role of PIN auxin transporters on lateral root initiation in patchy N environments in maize brace roots.

T8

Root hydropatterning: local water availability acts as a signal for lateral root initiation

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Plants grow in heterogeneous environments, and the mechanisms by which they perceive and integrate environmental signals into their growth and development are poorly understood. The root system must be especially sensitive to external stimuli as it navigates through complex soil environments, but it is unclear whether and how roots respond to environmental heterogeneity at the micro-scale. We model this environment by growing roots along the surface of agar media, exposing either side of the root to contact with a wet surface or air. Interestingly, lateral roots only develop on the contact side, while root hairs and aerenchyma only develop toward air. We have termed this developmental phenomenon as hydropatterning. The goals of this study are to determine what physical properties of the environment serve as cues for hydropatterning, the molecular mechanism by which these stimuli are perceived, and the downstream effects this has on the growth of the rest of the plant. We focus on the maize primary root, as its large size and the ease with which hydropatterning is observed make it an excellent model system to address these questions. We demonstrate that the local rate of water uptake by the root is a key signal for developmental patterning. Transcriptomic profiling of the two sides of the root using RNA-seq has identified several genes that may regulate lateral root initiation in response to this environmental signal. Genetic variation in hydropatterning has been observed in a forward genetic screen and in the maize nested association mapping (NAM) population; these resources will be used to test the role of hydropatterning in the field, and cloning of causal genetic loci is underway. Through these studies we hope to uncover the mechanisms underlying a novel moisture-associated developmental process, which may assist in designing crop varieties with optimal root traits for their local environment.

Funding acknowledgement: National Science Foundation (NSF)

T9

Genome-wide characterization of cis-acting DNA targets reveals the transcriptional regulatory framework of Opaque2 in maize

(presenter: Rentao Song <rentaosong@staff.shu.edu.cn>)
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Opaque2 (O2) is a transcription factor that plays important roles during maize endosperm development. Mutation of the O2 gene improves the nutritional value of maize seeds, but also confers pleiotropic effects that result in reduced agronomic quality. To reveal the transcriptional regulatory framework of O2, we studied the transcriptome of o2 mutant using RNA Sequencing (RNA-Seq) and determined O2 DNA binding targets using chromatin immunoprecipitation coupled to high-throughput sequencing (ChIP-Seq). The RNA-Seq analysis revealed 1,065 differentially expressed genes (DEGs) and 383 differentially expressed lncRNAs. The DEGs cover a wide range of functions related to nutrient reservoir activity, nitrogen metabolism, stress resistance, etc. ChIP-Seq analysis detected 1,686 O2 DNA binding sites distributed over 1,143 genes. Overlay of the RNA-Seq and ChIP-Seq results revealed 35 O2-modulated target genes. We identified 4 new O2 binding motifs; among them, TGACGTGG appears to be the most conserved and strongest. We confirmed that, except for the 16 kD and 18 kD zeins, O2 directly regulates expression of all other zeins. O2 directly regulates two transcription factors, genes linked to carbon and amino acid metabolism and abiotic stress resistance. We built a hierarchical regulatory model for O2 that provides understanding of its pleiotropic biological effects.

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

T10

Combinatorial Gene Regulation by R is Modulated by Small Molecule Interactions

(presenter: Erich Grotewold <grotewold.1@osu.edu>)
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Combinatorial gene regulation furnishes a mechanism by which a relatively small number of transcription factors control a much larger number of genes, with exquisite temporal and spatial expression patterns. The regulation of maize anthocyanin pigment biosynthesis by the interaction of C1/PL1 (R2R3-MYB) and R/B (bHLH) factors provides an outstanding system to understand plant combinatorial gene control. We recently discovered a novel mechanism involving C-terminal ACT domain that modulates the DNA-binding and protein-protein interaction activities of R. When R dimerizes through the ACT domain, the bHLH region cannot dimerize and R is tethered to DNA by its interaction with C1. In that configuration, which participates in the control of an R-regulated gene subset, the monomeric bHLH interacts with the chromatin factor, RIF1. However, when dimerization through the ACT is impaired, the bHLH is licensed to dimerize, recognizing canonical G-box DNA motifs characteristic of another set of R-regulated genes. The ACT domain is structurally similar to ligand-binding regions in many enzymes involved in amino acid and purine biosynthesis, where it participates in allosteric regulation. To understand what controls the dimerization of the ACT domain in R, we conducted a screen of a large number of phenolic compounds. Here, I will discuss the results of the screen and the implications for the control of flavonoid biosynthesis in maize.

Funding acknowledgement: National Science Foundation (NSF)

T11

The Maize TFome - development of a transcription factor open reading frame collection for functional genomics

(presenter: John Gray <jgray5@utnet.utoledo.edu>)
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Gene regulatory networks are central to all cellular processes. In plants, they help link molecular targets with agronomic traits of functional value including biofuel/biomass production, biomaterials, and nutritional health. Transcription Factors (TF) and co-regulators (CoReg) represent ~7% of the maize genome (~3000 genes) and are key regulators of plant metabolic function. To define the gene regulatory networks (GRNs) that regulate metabolism of maize phenolic compounds, we initiated The Grass Transcription Factor ORFeome Project (TFome).
We report the development and release of a publicly available maize TF ORF collection (TFome) of 2,034 clones corresponding to 2,017 unique gene models in recombination-ready vectors that make possible the facile mobilization of the TF sequences into a number of different expression vectors. The collection also includes several hundred co-regulators (CoREG), which we classified into well-defined families, and for which propose here a standard nomenclature, as we have previously done for TFs. Strategies were developed to overcome the limitations associated with cloning ORFs from a genome that remains incompletely annotated, with a partial full-length cDNA set available, and with many TF/CoREG genes lacking experimental support. This required, in many instances, combining genome-wide expression data with gene synthesis approaches. The strategies developed will be valuable for developing similar resources for other agriculturally important plants. Information on all the clones generated is available through the GRASSIUS knowledgebase (http://grassius.org/), and clones can be requested through the ABRC (http://abrc.osu.edu/). This resource and approach is expected to greatly accelerate the understanding of gene regulatory networks in plants. The maize TFome is now being employed to build a protein-DNA-interaction (PDI) network for the phenylpropanoid pathway. The first release of the Maize TFome has been described in a recent publication Burdo et al., 2014. The Plant Journal. 2014 80(2):356-66 This project is currently funded by NSF grant IOS-1125620 and previously by DBI-0701405.

Funding acknowledgement: National Science Foundation (NSF)

SESSION 6 - CYTOGENETICS & GENOMICS
Chair: Ruairidh Sawers Saturday, March 14. 8:15 AM – 10:15 AM

T12

Genomic features shaping the landscape of recombination hotspots in maize

(presenter: Wojtek Pawlowski <wp45@cornell.edu>)
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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. We generated a genome-wide map of DSB hotspots in several inbreds and hybrids of maize 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 recombination, such as centromeres and the pericentromeric regions. 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. The genic- and non-genic-region hotspots differ by several features, such as presence of a hotspot DNA sequence motif and specific histone modification patterns. Only genic-region hotspots contribute to the formation of COs. Comparing DSB maps of the B73, Mo17, and CML228 inbreds showed that some hotspots are shared among different inbreds whereas others are inbred-specific. Interestingly, examination of B73 x Mo17 and B73 x CML228 hybrids revealed presence of novel hotspots absent from the parental inbreds. Understanding recombination patterns will shed light on the mechanisms affecting dynamics of the maize genome and help devise methods for more efficient breeding.

Funding acknowledgement: National Science Foundation (NSF)

T13

De novo centromere formation in maize

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The centromere is the chromosomal site where the kinetochkore forms. It contains tandem repeats and retrotransposon sequences. The centromere has a mixture of chromatin including the epigenetic mark of the histone H3 variant CENH3 and conical histone H3. In maize, structural dicentrics have been found in which one set of sequences has become inactive and therefore these chromosomes are stable. Recently, many structural acentric fragments were documented that nevertheless were transmitted mitotically and meiotically. These de novo centromeres have a functional centromere and are formed at ectopic genomic regions. The DNA sequences are unique. We have discovered 30 de novo centromere formations from the offspring of centromere misdivision, pollen irradiation and other chromosomal manipulations. The CENH3 binding domain of de novo centroemre is typically smaller that the canonical centromere. DNA methylation and transcript analysis has been conducted on selected de novo centromeres.

Funding acknowledgement: National Science Foundation (NSF), NSFC

T14

Linking Chromatin Structure to Genomic Function through Differential Nuclease Sensitivity (DNS-seq) and Nucleosome Occupancy Mapping

(presenter: Hank Bass <bass@bio.fsu.edu>)
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Nucleosomes are the fundamental units of chromatin structure with important roles in genome function and regulation. In order to investigate maize chromatin dynamics, we developed nucleosome mapping assays using differential nuclease sensitivity (DNS) to discover a large new class of site-specific footprints that we distinguish as MNase sensitive (MSF) or MNase resistant (MRF). Using a microarray approach that sampled ~ 6% of the maize genome, we showed that the MSFs were located at transcription start sites (TSS), the 3' ends of genes, and in between genes, where they often mapped to conserved noncoding sequences (CNS) or transcription factor (Knotted1) binding sites (Vera et al., Plant Cell 2014). Furthermore, we found that the TSS MSFs varied in direct proportion to gene activity (RNA levels), suggesting these signatures may be predictive of transcription rates. Here we have extended this approach to the whole genome using DNS-seq with nuclei from B73 seedling shoot or root. We present new findings on the global distribution of MSFs and MRFs in the maize genome including further classification of these by location, underlying sequences, or proximity to other genomic and epigenomic features such as G-quadruplex elements, transposon insertion sites, and TF binding sites. Further classification of these footprints is expected to help resolve the complex and dynamic chromatin architecture in maize while focusing on regions of the genome where local chromatin structure is detectably altered by trans-acting factors or marks. We also show how conventional nucleosome occupancy mapping was used to uncovered drought-specific footprints. Together these genome analyses have revealed that chromatin structural dynamics coupled to genetic functions are resolved on a very small scale – often one nucleosome (~150 bp) or smaller. Experimentally, these hold great potential for bridging the growing layers of epigenomic information associated with chromatin structure and genome response in maize.

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

T15

Insights into the relationship between structural diversity and transcriptional diversity in maize

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Maize is a species with extensive sequence diversity. To further understand the maize pan genome, we have generated a comprehensive de novo assembly of the inbred line PH207 to complement the existing B73 reference genome assembly. B73 is an important founder line of the Stiff Stalk pool, while PH207 is an important founder of the Iodent Non-Stiff Stalk pool, both of which have been critical components of U.S. temperature maize germplasm. The PH207 assembly contains 132,022 scaffolds with an N50 of approximately 630 kb and a total assembly size of 2.1 Gb. Alignment of RNAseq reads from diverse tissues as well as conserved eukaryotic genes mapping approach (CEGMA) indicated that the gene space is well represented and comparable to the representation present in the B73 reference assembly. Comparative analysis between the B73 and PH207 genome assemblies revealed thousands of genotype specific genes and extensive expansion/contraction of gene families between the two genotypes, consistent with previous estimates based on transcriptome assemblies across 503 diverse inbred lines. We have also deeply resequenced 35 maize inbred lines and surveyed the genomic content of these lines. Interestingly, core genes that were present in all individuals had higher average expression levels across 80 tissues throughout development and were expressed in nearly all tissues, while genes present in a subset of the individuals showed more tissue/condition specific expression and on average had lower expression levels. Additionally, extensive genome content variation between heterotic groups was observed in the set of 35 resequenced inbred lines. A comparative analysis between the genomes and transcriptomes of diverse maize inbred lines and the role these differences may play in heterosis will be presented.

Funding acknowledgement: National Science Foundation (NSF), Department of Energy (DOE), Dow AgroSciences, DuPont Pioneer

T16

Comparing several maize reference genomes as a tool to discover conserved and variable key genetic elements in maize

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Extensive genome dynamics resulted in the vast accumulation of point mutations, indels and large structural variations during the evolution of Zea mays prior and after domestication. Those varied changes happen also in genic regions and affect gene content, gene order and predicted protein sequences. Consequently, the sequences most conserved among diverse maize inbred lines are expected to have indispensable role in maize life cycle. On the other hand, less conserved sequences have the potential to affect heterosis and impact the adaptation of maize to non-optimal growing conditions.
Using DeNovoMAGIC assembler we de-novo assembled three diverse inbred lines (PH207, CML247 and W22). By using deep coverage whole genome sequencing of Illumina short reads, the three new reference genomes have been produced with N50 of 0.3-1.7 Million bp. In addition, dozens of additional lines were de-novo assembled from lower coverage NGS data. The GenoMAGIC software was used to compare all genomes and to produce a broad database for maize gene alleles. Mining the database for all allelic variants for each gene, genes' PAVs, genes' CNVs, and synteny of gene order enable the better understanding of the basic maize gene set and the additional gene arsenal which could be exploited for both classical breeding and GM. The full genome assembly results and few key examples will be provided in details.

T17

Integrating tissue specific co-expression networks with NAM GWAS to prioritize candidate gene sets

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The maize nested association mapping (NAM) population has successfully identified hundreds of candidate loci for many complex traits. One challenge, however, is the interpretation of these large candidate lists. Unambiguous identification of a single causal locus is necessary for further understanding of the cellular mechanisms underlying the trait of interest. Identifying functional genes for complex traits potentially involving dozens of significantly associated SNPs quickly become unwieldy, even when using straightforward SNP-to-gene mappings such as two closest flanking genes. We have developed a novel method to address these problems by integrating datasets derived from co-expression networks to annotate and further filter candidate loci produced by GWAS studies.

To better understand grain composition, GWAS was performed on 19 different elemental components (Al, As, B, Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Mo, Na, P, Rb, S, Se, Sr, Zn) on over 55,000 maize kernels, resulting in strong associations across hundreds of different loci for each individual element. We employed SNP-to-gene mappings, including up to 4 flanking genes within 50kb only if flanking genes had co-expression interactions with candidate genes from different GWAS loci (trans co-expression). Significance of co-expression subnetworks identified by GWAS was assessed by bootstrapping, using random sets of equally sized SNP sets to estimate a null distribution. Using three different co-expression networks capturing expression variation from developmental tissues, the maize PAN genome, and 47 root tissues from diverse maize lines including many NAM founder parents, we found candidate genes associated with elemental accumulation were significantly enriched for strongly connected, co-expressed subnetworks. Furthermore, despite roughly doubling candidate sets with SNP-to-gene mappings, integrating co-expression networks effectively reduced candidate gene lists by two orders of magnitude. This demonstrates the value of combining multiple -omics datasets, each with non-overlapping sources of error, to accelerate functional gene discovery.

Funding acknowledgement: National Science Foundation (NSF)

T18

The maize *FASCIATED EAR3* gene reveals a new CLAVATA signaling system, and controls ear size and stem cell proliferation by signaling from differentiating cells.

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Vegetative shoot and inflorescence growth depends on pools of stem cells in the meristems. Shoot apical meristem (SAM) size is regulated during development by balancing stem cell proliferation with the incorporation of cells into primordia. Several *"fasciated ear" (fea)* mutants, with enlarged meristems, have been identified in maize. *THICK TASSEL DWARF1 (TD1)* and *FEA2* are homologous to the Arabidopsis leucine-rich-repeat (LRR) receptor-genes CLAVATA1 (CLV1) and CLV2, respectively. CLV1 and CLV2 act as receptors for CLV3, which is expressed in stem cells, and repress the stem cell-promoting transcription factor WUSCHEL. Maize has several additional *fea* mutants, many of which localize to seed row numbers QTLs, providing applications in crop improvement.

Here we present a phenotypic and molecular characterization of the *fea3* mutant, which shows over-proliferation of the inflorescence meristems, leading to fasciated ears and thick tassels. Double mutants of *fea3* with *td1* or *fea2* have additive and synergistic fasciated phenotypes, indicating that they act in independent pathways that converge on the same downstream target. We cloned the *fea3* gene using map-based cloning, and confirmed this by isolation of three additional *fea3* alleles from EMS mutagenesis. *FEA3* encodes a plasma membrane-localized LRR receptor-like protein that is expressed in the organizing center of the SAM and in leaf primordia. Remarkably, maize WUSCHEL expression spreads downwards in *fea3* mutants, which is strikingly different from its response in *CLV* mutants. *fea3* mutants showed reduced sensitivity to the peptide ZmFCP1. ZmFCP1 is expressed in leaf primordia, suggesting that it serves as a signal from differentiating cells to repress meristem growth via the FEA3 receptor.

Our results indicate that FEA3 functions in a new pathway for stem cell control that is spatially distinct from the known CLV receptors, using a different peptide signal. This signaling system appears to be universal, as we have found orthologs in *Arabidopsis.*

Funding acknowledgement: National Science Foundation (NSF), DuPont Pioneer

T19

Natural variation of maize shoot apical meristem morphology

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Through the combined activities of stem cell maintenance and leaf and branch organogenesis, the maize shoot apical meristem (SAM) determines the number and position of all lateral organs in the above ground plant. Decades of genetic research have identified complex, interactive networks regulating SAM function and morphology, although information regarding SAM morphological variation in natural populations and diverse inbreds within the genus Zea is scarce. To examine the genetic architecture of morphological microphenotypes in the maize SAM, we employed a genome-wide association study (GWAS) among a diverse panel of 380 inbred lines. Utilizing a high-throughput microscopy/image-processing pipeline and a novel 1.2-million-SNP dataset comprising RNAseq-generated and publically available genotypes, we identified several high-quality candidate genes associated with variation in the maize SAM morphospace. Although the majority of these GWAS-derived SAM candidate genes were previously unpredicted to affect SAM structure or function, bioinformatic and molecular analyses validated the correlation between several trait-associated SNP's (TAS) in candidate genes and SAM size/shape. *In situ* hybridization analysis of a predicted auxin import protein showed TAS-correlated, spatiotemporal differences in transcript accumulation patterns that also correlated with SAM size. Cell counting and size estimation by confocal microscopy showed a correlation between natural variation in a predicted brassinosteroid-receptor-like kinase and increased SAM cell size. Ongoing studies suggest that previously unexpected auxin import and cell size regulatory pathways interact with SAM function and contribute to SAM morphological variation.

Funding acknowledgement: National Science Foundation (NSF)

T20

Cytokinin hypersignaling reprograms maize proximal-distal leaf patterning

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Organized, patterned growth is essential for normal multicellular development. We are using the maize leaf as a model to understand the molecular signals contributing to pattern formation. The maize leaf is composed of four compartments – the sheath, ligule, auricle and blade – organized along the proximal distal growth axis. The semi-dominant, leaf-patterning, maize mutant *Hairy Sheath Frayed 1* (*Hsf 1*,is characterized by leaves with outgrowths of proximal tissues (sheath, ligule and auricle) emanating from the distal blade. Our analysis of *Hsf1* indicates specific missense mutations in the cytokinin receptor gene, *Zea mays Histidine Kinase 1* (*ZmHK1*), cause this phenotype. These mutations cause the receptor to hypersignal cytokinin (CK) in developing leaf primordia giving rise to outgrowths with proximal identity (prongs) in the distal blade. To gain further insight into how CK hypersignaling reprograms leaf patterning, we used laser microdissection RNA-Seq analysis on developing prongs. Transcriptomic analysis revealed about 900 differentially expressed (DE) genes in *Hsf1* prong margins compared with wild type margins. Among the DE genes, we find an over-representation of specific transcription factors and hormone pathway genes that are known to control the formation of lateral organs like leaves, lateral branches, leaflets, and the ligule. To test the idea that organogenesis genes contribute to prong formation, we have combined *Hsf1* with mutations in some of the DE genes with known functions and analyzed their epistatic interactions. Double mutant plants showed enhancement of the *Hsf1* phenotype, confirming the predicted role the DE genes play in prong and lateral organ formation. These results suggest that CK hypersignaling in the distal blade triggers the misexpression of downstream pathway genes, which specifies a new type of lateral organ with proximal identity – the prong. Our study is significant because leaf patterning controls leaf architecture and overall plant morphology, which determine grain and biomass yield.

Funding acknowledgement: National Science Foundation (NSF)

T21

A novel DICER-LIKE1 pathway in maize buffers for loss of DICER-LIKE4 in tasiR-ARF biogenesis

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Dicer enzymes function at the core of RNA silencing to defend against exogenous RNA, or as an endogenous mechanism of gene regulation. Plant DICER-LIKE4 (DCL4) performs dual functions, acting in antiviral defense, as well as in development via the biogenesis of tasiR-ARFs. These small RNAs play an essential role in the grasses and act to spatially define the expression domain of AUXIN RESPONSE FACTOR3 (ARF3) transcription factors. In maize, mutants affecting early steps in ta-siRNA biogenesis, e.g. *leafbladeless1* (*sgs3*), *ragged seedling2* (*ago7*) and *rdr6*, develop severe leaf polarity defects and frequently arrest shortly after germination. However, such an essential role in development is contradictory to DCL4's need to diversify as an antiviral defense protein. Indeed, evidence of recurrent selection at the Dicer PAZ domain is a hallmark specifically of plant DCL4 enzymes, and particularly in the monocots. To address how DCL4 balances its role in development with the requirement to undergo recurrent adaptive changes as an antiviral defense protein, we screened mutagenized maize populations for plants exhibiting adaxial-abaxial leaf polarity defects that map near *dcl4*. We found that, in contrast to other tasiR-ARF biogenesis mutants, *dcl4* null alleles condition an uncharacteristically mild phenotype, correlated with normal expression of select *arf3* targets. Loss of DCL4 activity yields a class of 22-nt tasiR-ARF variants associated with the processing of *arf3* transcripts into 22-nt secondary siRNAs by DCL1. Our findings uncover the presence of a novel DCL1-dependent siRNA pathway that mitigates the otherwise adverse developmental effects of *dcl4* mutations. Furthermore, this novel pathway has implications for DCL4's role in antiviral defense by reducing the selective constraints on DCL4 and allowing it to diversify in response to viral attack.

Funding acknowledgement: National Science Foundation (NSF)

T22

The SCAR/WAVE complex polarizes PAN receptors and promotes division asymmetry in maize

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Specialized cell types and new cell lineages in plants are produced via asymmetric cell division. In maize, stomatal complexes consist of two guard cells each flanked by a subsidiary cell. Subsidiary cells arise via asymmetric divisions of subsidiary mother cells (SMCs), which polarize toward adjacent guard mother cells (GMCs). Previous work showed that two receptor-like kinases (PAN2 and PAN1) and the small GTPase ROP promote mother cell polarity and subsequent division asymmetry in SMCs. PAN proteins become polarized prior to asymmetric cell division, followed by formation of a dense actin patch, nuclear migration, and eventual asymmetric cell division. Loss of function mutations in *pan1* and *pan2* genes result in aberrant subsidiary cells, due to a failure in SMCs polarization. Mutations in *brk1* and *brk3* cause similar subsidiary defects, and thus we set out to establish the role of BRK proteins in SMC polarization. BRK1 and BRK3 are components of the SCAR/WAVE regulatory complex, which activates the actin-nucleating ARP2/3 complex. Given the known interactions between BRK, SCAR, actin and ROPs in other organisms, we hypothesized that PAN1-dependent recruitment of ROPs would in turn activate BRK, resulting in actin patch formation. Contrary to predictions of this hypothesis, BRK1 localizes within SMCs at GMC contact sites earlier than PAN1 and PAN2. Furthermore, polar localization of PAN1 and PAN2 is disrupted in *brk1* and *brk3* mutants, implicating the SCAR complex in polar localization of PAN LRR-RLKs. These findings demonstrate that SCAR complex subunits function upstream of PANs in SMC polarization and establish the SCAR complex as the earliest acting component of the SMC polarity pathway.

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T23

Macroscopic microscopy: elucidating mechanisms of pathogen resistance for genes associated with quantitative defense

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Our understanding of the genetic architecture of quantitative traits has rapidly advanced over the past decade, leading to an expansive catalog of genomic regions associated with phenotypic variation. Lagging behind is our determination of the underlying causal variants and an understanding of how they function to produce observed, macro-level phenotypes. The DR Maize (Disease Resistance of Maize) project is discovering and validating genomic variants for resistance to quantitative defense against fungal foliar pathogens using a combination of GWAS, breakpoint analysis, mutant screens, expression analysis, and transformational validation. Simultaneously, this project aims to characterize gene function by 3D microscopy on infected tissues of isogenic lines that contrast for natural and induced allele effects. Here, we briefly summarize our progress on gene discovery and validation while presenting more in-depth information about the development of an imaging platform for studying pathogenesis and gene mechanisms associated with disease resistance. This includes experimentally optimized procedures for sampling, fixing, clearing, staining and imaging, as well as computational methods for image management, manipulation, segmentation, and quantification. Using this platform to examine disease development incited by two fungal pathogens of maize, we have observed some features of pathogenesis that to our knowledge have not been previously described. Furthermore, to characterize the distributional structure of pathogen features and pathogenesis events associated with disease resistance, we are quantifying microscopic information across the macroscopic scale of leaf tissues; the latest results on this will be presented. Working with two fungal pathogens of maize and other evidence suggests that the platform is extensible for examining an array of plant/pathogen systems, as well as a general platform for investigations on quantitative tissue and cellular biology.

Funding acknowledgement: National Science Foundation (NSF)

T24

Patterns of demography and selection since maize domestication

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The domestication of maize from the wild grass teosinte began in central Mexico approximately 9,000 years ago. Previous genetic studies focusing on coding sequences have demonstrated a loss of diversity due to a domestication bottleneck, but technological limitations have precluded independent estimation of the parameters of the bottleneck. Here, we use whole-genome sequence information from 13 teosinte and 23 landrace maize inbred lines, collected across the range of both taxa, to better understand the demography of domestication. We observe differences in the site frequency spectrum between coding and non-coding regions, implying an interaction between selection and demography in determining patterns of diversity. By comparing genetic diversity surrounding synonymous and nonsynonymous substitutions we find evidence that classical hard-sweeps were rare in both maize and teosinte. A stronger reduction in pairwise diversity near genic regions is seen for teosinte than for maize, but when we restrict diversity calculations to rare alleles, the opposite pattern is observed. This implies that purifying selection may have been weaker in maize shortly after the bottleneck, but subsequent population expansion has led to purifying selection in maize having a stronger effect. Together, these findings allow us to elucidate details of the domestication process and its lasting effects on genetic diversity in the maize genome. These results have implications for understanding the functional consequences of diversity and may guide future strategies for further maize improvement.

Funding acknowledgement: National Science Foundation (NSF)

T25

Dissecting the regulatory divergence between maize and its progenitor, teosinte

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The regulation of gene expression is theorized to play an important role in species evolution. The cloning of a few key genes for the major morphological changes during maize domestication has highlighted the importance of gene expression regulation in maize domestication from its ancestor, teosinte. Here, we report a comprehensive assessment of gene expression variation by sequencing the transcriptome of a large maize-teosinte experimental population. The genome-wide mapping identified 19,854 expression quantitative trait loci (eQTL) for 14,866 genes, capturing an unprecedented range of expression variation. Consistent with prior studies, our data indicated that domestication has strongly altered the global expression pattern, with more frequent up-regulation of genes in maize as compared to teosinte. We showed that genes in secondary metabolism are more likely subject to local regulatory modifications, reflecting the importance of secondary metabolic diversification in plant speciation. We demonstrated a striking example of this feature that an “operon-like” gene cluster for the biosynthesis of benzoxazinoids, compounds conferring broad pest and pathogen resistance, is under coordinated local regulatory modifications. A total of 25 significant distant-eQTL hotspots were identified, with their targets significantly enriched in specific functional categories. We demonstrated how the regulatory relationships between putative regulator and targets have evolved using a hotspot involved in glycolysis as an example. These results provide novel insights of gene expression adaptation during maize domestication.

Funding acknowledgement: National Science Foundation (NSF), National Natural Science Foundation of China

T26

Multi-parent and intermating population design effects on genetic mapping resolution of major color genes

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Linkage populations with multiple parents can provide improved locus resolution in genetic mapping. This improved resolution may be enhanced further through intermating individuals prior to inbreeding. The maize (*Zea mays* L.) population developed here adopts a series of designs with both of these strategies. Combined, an unprecedented 1,211 individual mapping population with 107,309 genetic markers allows empirical comparisons of the effects on genetic mapping resolution and accuracy. Using association mapping methods we identified two epistatic candidate loci co-localizing to known mutants for control of the blue aleurone, believed to be under complex genetic control. These results suggest that these two genes largely control blue aleurone expression in improved breeding lines. However, in both smaller sets of four way individuals and in bi-parental crosses, one or both genes were not always detected or were detected in the wrong location. Known Mendelian genes for yellow endosperm and red cob color showed similar but less dramatic losses in reducing population size. Unexpectedly, multiparent populations with four founders and intermating resulted in minimal enhancement of locus resolution when compared with conventional two-way crosses; the effect of increased population size was most beneficial. This new population provides a design that can be used to better understand limitations in genetic mapping accuracy and resolution and ultimately better understand effective genetic recombination. We conclude that much larger linkage mapping populations than are routinely used are necessary for accurate detection of traits with even simple inheritance. Furthermore, we discuss that the current software and approaches for genetic linkage map construction can be unsuitable for large multi-parent populations with large marker sets.

Funding acknowledgement: United States Department of Agriculture (USDA)

SESSION 10 - TRANSPOSONS & EPIGENETICS
Chair: David Braun Sunday, March 15. 10:20 AM – 11:00 AM

T27

mop1 impacts the maternal contribution to maize seed

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Seeds develop by double fertilization in which the diploid central cell and haploid egg are fertilized by a haploid sperm, giving rise to triploid endosperm and diploid embryo, respectively. Seed lethality is observed when the parental contribution to the endosperm differs from a 2:1 maternal:paternal genome copy ratio. Interploidy crosses, those between individuals with different genome copy numbers, results in seed death with categorically distinct terminal seed phenotypes. We have identified natural variation affecting the frequency of terminal seed phenotypes and attempted to map QTL using the IBM (intermated B73 x Mo17 recombinant inbred lines) as ear-parents crossed to tetraploid pollen parents. Terminal seed phenotypes had high heritability, but no major effect QTL were identified. In F1 x tetraploid crosses using B73 x Mo17 reciprocal F1 hybrids as ear parents, we observed a grand maternal effect that was not cytoplasmic inheritance. We hypothesized that parental epigenetic state contributes to the dosage-sensitive nature of the developing endosperm. Small RNA (sRNA) are implicated in gene expression, gametophyte development, genomic protection from transposable elements as well as disease and stress response. We have evaluated DNA methylation and sRNA mutants in paternal excess crosses. Higher seed lethality in the sRNA pathway *mop1-1/+* and *Mop2-1/+* heterozygotes crossed to tetraploids was observed but no lethality increase in crosses to methylation mutants. Surprisingly, triploid individuals resulting from *mop1-1/+* x tetraploid crosses show adult plant phenotypes not observed in the parental generation nor in wild type x tetraploid crosses. This indicates that the sRNA from the female gametophyte impacts the epigenome at fertilization affecting seed survival and having long term consequences for plant development in maize paternal excess crosses.

Funding acknowledgement: United States Department of Agriculture (USDA)

T28

RNA-directed DNA methylation in suppression of intronic transposons: minimizing collateral damage on host gene expression?

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A key challenge in genome defense is to selectively target repetitive DNA for suppression without compromising desired gene expression. This challenge is particularly acute in the case of genes that host transposons in their introns, where transposon suppression must be compatible with host gene expression. We have found that RNA-directed DNA methylation (RdDM) is a widespread mechanism of intronic transposon suppression in maize. In contrast to expectations, however, mutant analysis revealed that loss of RdDM resulted in loss rather than gain of host gene expression. We have also identified several chromatin features associated with intronic transposons, including the histone modifications H3K9me2, H3K27me2 and H3K27me3. We propose a model in which RdDM represses intronic transposons in a way that prevents their being targeted by other genome defense mechanisms that are less compatible with host gene expression.

Funding acknowledgement: National Science Foundation (NSF)

T29

Ds mutagenesis and a *de novo* W22 genome sequence

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Targeted mutagenesis continues to drive the dissection of gene function in maize, and endogenous transposable elements are a key resource for those analyses. Nearly half of annotated maize genes contain a publicly available transposon insertion, but many of those insertions do not knock out gene function and a large majority of genes still have no publicly available mutants. Here we demonstrate the utility of the transposable element *Ds* for regional mutagenesis to mutate genes of interest. Numerous *Ds* elements were used to conduct both forward and reverse genetic screens, culminating in over 130 insertion alleles in 30 different gene targets. Germinal *Ds* insertions were isolated in single-copy genes, double- and triple- mutants were also recovered from a tandem gene cluster, and duplicate factor mutants were produced in combination with existing resources. Furthermore, footprint alleles generated during *Ds* excision were identified using a high-throughput, PCR-based approach to generate additional allelic diversity. From the large number of donor *Ds* elements now mobilized into targets at various genomic distances, we summarize the efficacy of *Ds* insertional mutagenesis for *Ds*-target gene intervals up to approximately 500 kb. In public seed stocks, available from the Co-op Stock Center or the project (http://acdstagging.org/), 75% of all genes are within this range of an existing *Ds* in an inbred W22 background. New *Ds* transpositions are being mapped using high throughput sequencing and all insertions will be placed to a high quality, de novo assembly of the W22 genome (conducted in collaboration with NRGene), further improving the utility of the resource. Preliminary data analyses indicate an N50 of 1.5 Mb, suggesting that this assembly ranks second only to B73. The sequence will be publicly available after QC analysis. Here we present a description of the maize W22 genome and discuss the value of the extensive endogenous *Ds* resources currently available.

Funding acknowledgement: National Science Foundation (NSF)

Poster Abstracts

P1

A Preliminary Freezing Tolerance Screen in the Perennial Grass Genus Tripsacum, Which Is Closely Related to Maize

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Freezing temperature poses a severe challenge to many biochemical and physiological processes in plants. Freezing tolerance refers to the biochemical changes that occur in response to low temperature. The expression of freezing tolerance genes increases during periods of moderately low temperature exposure in a process called cold acclimation. Grass species in the two genera *Tripsacum* and Zea differ in their ability to withstand freezing temperatures. Even though both grass genera originated in the tropics, *Tripsacum* species are perennial and can overwinter in a dormant state, while maize cannot survive prolonged freezes. The *Tripsacum* genus diverged from the *Zea* genus fewer than 1.2 million years ago, prior to the domestication of maize (Ross-Ibarra et al., 2009). *Tripsacum* and maize share most of their gene content, yet the basis for freezing tolerance in *Tripsacum* and freezing sensitivity in maize remains largely unknown. Here, we describe a preliminary freezing tolerance screen using 161 *Tripsacum* offspring from crosses between *Tripsacum* accessions from the northern and southern United States. Plants were exposed to room temperature, transferred to 5 °C growth chambers for two weeks during cold acclimation, and then transferred outdoors during the winter. Leaf tissue was collected at each stage for mRNA-sequencing using 3'-UTR sequence tags. Plants were genotyped using Genotyping-by-Sequencing and phenotyped at each stage of cold exposure. This pilot study will reveal the extent of freezing tolerance in diverse *Tripsacum* accessions, cold-induced gene expression dynamics, and single nucleotide polymorphisms that are associated with freezing tolerance.

Funding acknowledgement: United States Department of Agriculture (USDA)

P2

An integrated system-wide maize atlas: from transcriptome to proteome networks

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Integrated molecular atlases make possible systems biology approaches aimed at understanding biological phenomena. Using RNA-seq and quantitative mass spectrometry we generated an atlas comprised of 41,272 transcripts, 18,646 proteins and 32,000 phosphopeptides, quantified across maize development. Analysis of the atlas has revealed complex spatiotemporal patterns of gene activity. For example, there is poor correlation between protein and mRNA levels and for many of the most abundant proteins there is little to no detectable cognate mRNA. The atlas has also enabled generation and interrogation of a number of different types of regulatory networks including mRNA and protein co-expression networks, a gene regulatory network (GRN) and a novel predicative kinase-substrate network. Together, these studies highlight the complex interplay of transcriptional, translational and post-translational events in dynamically remodeling the proteome.

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P3

ANGSD-wrapper: scripts to streamline and visualize NGS population genetics analysis

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The advent of highly multiplexed sequencing has opened a number of exciting avenues for evolutionary biologists. One of the powerful approaches enabled by inexpensive sequencing is the ability to sequence a large number of individuals, each to relatively low sequencing depth. However, this approach also presents statistical challenges in the analysis of low coverage data. The software ANGSD [3] and related programs [2] were developed to deal with low coverage sequence data. Rather than call genotypes at variable sites, ANGSD performs a number of population genetic analyses on genotype likelihoods, including estimation of the population mutation rate θ, the site frequency spectrum, neutrality tests, inbreeding coefficients, and population structure. ANGSD has already been used in several studies to analyze genome sequence data [1] [4]. However, ANGSD requires considerable familiarity with command line tools and remains inaccessible to many biologists that are not from a computational background. Here we present a software package that aids in the preparation of analy- ses for ANGSD and provides interactive graphing software implemented in R [5] and Shiny [7]. ANGSD-wrapper simplifies multistep analyses such as calculating Tajima's D into a single step. Users supply all the needed in- formation in a single configuration file (Figure 1), and after ANGSD has fin- ished calculations, ANGSD-wrapper provides interactive graphing of the results (Figure 2). ANGSD-wrapper is available on github: https://github.com/ arundurvasula/angsd-wrapper.

P4

Araport: the Arabidopsis Information Portal

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Araport, the Arabidopsis Information Portal, (https://www.araport.org), is an open-access online community resource for plant research funded by NSF and BBSRC that started in 2013. The goal of Araport is to provide users with a single access point ("one-stop-shop") to Arabidopsis-related information through data federation, collecting data from diverse and geographically dispersed centers through state-of-the-art web technologies. Because of this Araport data model, users can now access not only TAIR10 gene annotation but in addition data from UniProt (protein), BAR (expression), EPIC-CoGe (epigenomics), IntAct (interaction networks), ATTED-II (coexpression), PubMed (literature) and many other resources. Araport inherited from TAIR the responsibility of providing free access to up-to-date structural and functional annotation for the Col-0 genome and will release an update - Araport11 - later this year.

The vision of Araport is to grow with the research community by providing an extensible framework for incorporation of new data sources and creation of user interfaces to consume them. Future plans include incorporation of 1001 genomes polymorphisms, tissue-specific RNA-seq expression, seedstock, community annotation, and other data types.

For software architecture, Araport uses a data warehouse to provide a searchable index of sequence-associated data, as well as data federation to deliver in-depth information at run time. Araport incorporates and integrates software from GMOD including InterMine, JBrowse, GBrowse, WebApollo, Tripal, and Chado.

Funding acknowledgement: National Science Foundation (NSF), UK Biotechnology and Biological Sciences Research Council

P5

Assembling maize inbred CML247: the maize pan-genome takes off

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Maize has a complex genome, exhibiting highest amounts of structural variations (SVs) among the major crop species. For example, only about half of the genome is shared between any two maize varieties. The single B73 reference genome is insufficient to represent all of the genomic content of maize, which leads to underrepresented diversity and spurious SNP calls during variation discovery. Hence, a pan-genome, including multiple reference genomes of representative maize varieties, is needed to capture those untapped genetic variations and improve the quality of current variation discovery. Initializing the maize pan-genome construction, we recently started sequencing and assembling maize inbred line CML247 as a pilot project, to optimize maize de novo assembly approach by testing different sequencing platforms and assembly algorithms. Currently, the NRGENE approach, DISCOVAR de novo, Nanopore MinION device, and BioNano Irys System are being tested. In addition, many representative maize de novo assemblies are being collected from our collaborators. A set of ultrahigh density genetic markers (8.1M) is used to assess the quality of assemblies. Now we are constructing the whole genome alignment database of maize and the pan-genome based variation discovery will be performed.

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P6

Assessing the prevalence and diversity of G-quadruplexes in regulatory regions of maize genes

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G-quadruplexes (G4) occur at sites associated with gene regulation in maize and are disproportionately associated with genes that encode biochemical pathways involved in energy status, hypoxia, low sugar, and nutrient deprivation (Andorf et al., JGG 2005 41(12):627-647).We wish to determine: (1) whether the distribution of G4 elements varies across diverse germplasm and (2) whether and how stacking alleles of genes that have a G4 motif modulates the stress response. As a first step, we investigated the distribution of G4 motifs in Mo17 versus B73. Here we describe the results of that comparison and outline next steps.

P7

Automated construction of high-quality LTR exemplars from plant genomic sequences

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Long terminal repeat (LTR) retrotransposons represent the largest component in most plant genomes. Construction of the LTR library becomes one of the crucial tasks in genome annotation projects, as well as understanding the evolution of genome size across species. De novo discover of LTRs computationally has been achieved based on their highly structured characteristics, however the output may not be always ready for genome annotation, often due to false positives and significant redundancy. We observed that most false positives generated by de novo LTR annotation softwares are due to the vicinity of highly similar repetitive elements other than LTR retrotransposons (eg. LINE, SINE, solo-LTR, tandem, etc). By taking advantage of these features, we developed a package that distinguishes true and false LTRs, as well as reduces redundancy. When testing in the rice (Oryza sativa) genome which has a well curated TE library, we obtain 85% (1332) LTRs with perfect structures out of 1567 pass-criteria candidates. Also, the constructed LTR exemplar of rice genome had demonstrated 93.06% sensitivity and 99.17% specificity. With tests in more rice genomes (93-11, Kasalath, IR64 and DJ123), the maize (Zea mays) genomes (B73 and Mo17) and the sacred lotus (Nelumbo nucifera) genome, our package was shown well adaptable and flexible to construct LTR exemplars in plant genomes.

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P8

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 (genome annotations) 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 the beneficial and 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, both deleterious and beneficial.

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P9

Building a phylogenetic framework for comparative genomics in Andropogoneae

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The tribe Andropogoneae includes a number of economically and ecologically important species, such as *Zea mays*, *Sorghum bicolor*, *Miscanthus* spp., *Saccharum* spp., and *Andropogon gerardi*. Due to the high value of this group, a number of genomic resources have been created including genomes for maize and sorghum, upcoming genomes in *Miscanthus* and *Saccharum*, and multiple transcriptomic datasets. Despite these efforts, the capacity for comparative genomics in the group remains constrained by poorly understood relationships and large gaps in sampling of phylogenetic diversity. Here, we present a well-resolved whole chloroplast phylogeny consisting of more than 100 samples representing over 60% of the total generic diversity of the tribe. We demonstrate that much of the genome sequencing in the Andropogoneae is focused in a relatively small clade comprising *Miscanthus*, *Saccharum*, *Sorghum*, *Sarga*, *Cleistachne*, and *Hemisorghum*. We also show that there has been hybridization between the genera *Miscanthus* and *Saccharum*. The *Zea*/*Tripsacum* clade, where other major genomic resources are available, diverged early in the history of Andropogoneae and are on a relatively long branch prior to their diversification, a possible consequence of the shared paleopolyploid event of the group. We highlight regions of the Andropogoneae phylogeny that would be beneficial for increased genomic sampling to provide maximum leverage with existing grass genomes.

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P10

Computational exploration of the metabolic network of surface lipid production on maize silks

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The maize silk cuticle provides protection against abiotic and biotic stresses encountered by silks that have emerged from the encasing husk leaves into the environment. Silk cuticular lipids are comprised primarily of hydrocarbons and fatty acids, and also include aldehydes and alcohols ranging from 16-35 carbons in length. The metabolic reactions (i.e. the metabolic network) responsible for the production of these silk surface lipids is not well defined. To dissect the metabolic network for surface lipid accumulation, we have first profiled surface lipids along the lengths of silks among the maize inbreds B73 and Mo17, and their reciprocal hybrids, and we have demonstrated that the complex arrays of surface lipids (approx. 120 metabolites) vary across silk development and among genotypes. To incorporate these surface lipid profiles into a model of the metabolic reaction network, we are integrating two computational and statistical approaches. First, the computational tool, BNICE (Biochemical Network Integrated Computational Explorer) is being used to generate all possible biochemical reactions from our surface lipid metabolites, based on a given set of enzyme reaction rules, thus providing information for biologically unknown reactions and/or undetectable reaction intermediates. Second, the Graphical Lasso algorithm is being applied to identify correlations among amounts of surface lipid metabolites, by estimating the covariance matrix comprised of all detected metabolites. Correlation among specific metabolites may suggest precursor-product relationships in the surface lipid metabolic network. We will discuss the importance and implications of building metabolic network models that account for variations in metabolite accumulation along the length of the silk and among genotypes that have diverse surface lipid metabolomes.

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P11

Consistent Non-Random Patterns in Nucleotide Base Composition across Genome-Wide Sequence Polymorphisms

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DNA base composition is a fundamental genome feature. However, the evolutionary pattern of base composition and its potential causes have not been well understood. Here, we report findings from comparative analysis of base-composition patterns at the whole-genome level across 2,210 species, polymorphic-site level across 8 population comparison sets, and mutation-site level in 12 mutation-tracking experiments. We first demonstrate that base composition follows the individual-strand base equality at the genome, chromosome, and polymorphic-site level. More intriguingly, clear separation in base-composition values across polymorphic-sites was consistently observed between basal (ancestral) and derived (bottlenecked) groups, suggesting a common mechanism for this non-random pattern. Individuals in the derived groups show an A&T-increase/G&C-decrease pattern compared with the corresponding basal groups. Analyses of spontaneous and induced mutation experiments indicated the same A&T-increase pattern. With base-composition values across polymorphic-sites as a genome phenotype, genome scans with human 1000 Genomes and HapMap3 data identified a set of significant genomic regions enriched with Gene Ontology (GO) terms for DNA repair.

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P12

Detection of induced mutations in the resequenced genomes of 600 EMS-mutagenized Sorghum BTx623 individuals

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Sorghum is a valuable panicoid grass species adapted to high temperature arid environments and poor soil nutrition. It an important source of food, feedstock and biomass production with tremendous potential. To expedite gene function discovery in sorghum, 600 sorghum lines mutagenized by EMS were sequenced at relatively low depth (6X average). We developed an effective and high throughput method for detecting EMS-induced mutations in the resequenced genomes of the 600 mutagenized sorghum individuals. We detected a total of 1,268,926 SNPs and an average of 2,115 SNPs per individual, with an estimated mutation frequency of one mutation per 344Kb. EMS almost exclusively results in G to A mutations, and our novel filtration procedure results in 98 and 99 percent of the SNPs were G to A and C to T substitutions in the whole genomes and protein-coding regions respectively. We also predicted a total of 4,011 high impact mutations in 3,605 distinct genes of which 2,860 were stop codon gained mutations. Similarly we predicted a total of 56,087 moderate impact mutations affecting 23,128 distinct genes. Overall we estimated an average of six high impact and 93 medium impact mutations per individual. We anticipate plant breeders, geneticists and the general scientific community would find this genetic variation, and our cost and compute resource efficient strategy for eliminating false positives, to be very useful for crop genomics and improvement

Funding acknowledgement: Bill and Melinda Gates Foundation

P13

Differential gene expression of maize aleurone and starchy endosperm cells at late developmental stages.

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The cereal endosperm consists of starchy endosperm (Ste) cells, which accumulate storage proteins and starch, the peripheral aleurone (Ale) cells, which mobilize these storage compounds during germination, and transfer cells, which are in contact with the embryo. Ale cells accumulate storage compounds such as proteins, lipids, minerals, as well as phytic acid, which is an anti-nutrient that prevents mineral absorption, and ferulic acid, carotenoids, and flavonoids, which have been independently identified as antimutagens, antioxidants, and anticarcinogens. By analyzing the genes expressed in maize Ste and Ale cells, we want to identify main metabolic and cellular trafficking pathways related to both development and nutrional properties of the maize endosperm. Here we present a systematic comparison of the transcriptomes of the Ste and Ale of maize B73 at 18 and 22 days after pollination (DAP). Illumina sequencing generated 5-25 million reads per tissue sample, with 60%-92% mapped to the reference genome. Using paired tests of EdgeR, we identified 153 genes in Ale and 457 genes in Ste that are differentially expressed at 18 vs. 22 DAP. Similarly, we identified 4,818 genes at 18DAP and 5,261genes at 22DAP that are differentially expressed between Ale and Ste. We investigated the function of these differentially expressed genes (DEGs) by summarizing their gene oncology (GO) terms and conducting GO enrichment tests. Additionally, we explored the DEGs involved in known metabolic pathways, signaling, and cellular trafficking using the Mapman tool. To investigate tissue specific expression patterns of the DEGs, we performed K-means clustering based on their expression levels in Ale, Ste, basal leaf meristem, and mature leaf. We show an overview of distinct metabolic pathways, transcription factors, and transposon active in Ste and Ale. This study is important for the understanding of maize endosperm development and for developing strategies to improve the cereal nutritional quality.

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P14

Discovery and Mapping of Presence-Absence Variants (PAV) in the Founders of the Maize NAM Population

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Genomic structural variation is increasingly being recognized as a major contributor to phenotypic variation across species. In plants structural variants contribute to traits including soybean resistance to soybean cyst nematode, barley boron tolerance and flowering time, wheat dwarfism and flowering time, and maize aluminum tolerance. Presence-absence variants (PAV), a form of structural variation where a DNA sequence is present in one genome but entirely missing from another, has been found to contribute to a number of genetic diseases in humans However, the association between PAV and phenotype is still being explored in plants with very few documented examples, including flavor quality in strawberries and submergence tolerance in rice. We enabled global explorations of the association between PAVs and phenotypic variation by conducting RNA-sequencing on the 27 inbred founders of the maize Nested Association Mapping (NAM) population. This exploration identified thousands of putative expressed PAVs that are absent from B73, the maize reference. In collaboration with Roche-Nimblegen these putative PAVs were used to design a Zeanome sequence capture array. Using this array, sequence capture of the NAM founders identified ~12,000 genomic PAVs that are absent from B73. Characterization of these PAVs shows that many have similarities to characterized proteins. Using the Fluidigm BioMark Dynamic Array, we have genotyped presence-absence variation for a subset of PAVs and genetically mapped them in the NAM RILs. This resource will allow us to test for associations between PAVs and with phenotypic variation in yield-related traits.

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P15

Dissecting evolutionary relationships of known genes with minimum spanning haplotype networks from maize HapMap3.1

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Understanding the genetic loci responsible for phenotypic effects can help breeders more efficiently make genetic gains for traits with known loci of high effect. However, this does not necessarily help identify the correct germplasm to focus breeding efforts on. We present haplotype networks (minimum spanning networks) as a tool for understanding the evolutionary relationships between haplotypes at loci responsible for flowering time and carotenoid and tocochromanol compounds. These networks are presented in light of effect estimates for phenotypes derived from GWAS and joint linkage analyses in the Maize Diversity Panel and the Nested Association Mapping population, respectively.

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P16

Dissecting Meiotic Recombination based on Tetrad Analysis by Single Microspore Sequencing in Maize

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Meiotic recombination drives eukaryotic sexual reproduction and the generation of genome diversity. An ideal way to study the mechanisms of homologous recombination is tetrad analysis examining the four chromatids resulting from a single meiosis. Here, we developed a simple method to isolate the four microspores from a single tetrad in maize for whole genome sequencing. Comparing genotypes of the four chromatids, recombination (Crossover and Non-Crossover) could be detected accurately. A high resolution recombination map was constructed from 24 tetrads by using 599,154 high-quality SNPs. The results reveal that crossovers were unevenly distributed across the genome, and more likely to occur in the genic than intergenic regions, and were especially common in the 5' and 3' end regions of annotated genes. Gene conversions were directly detected and the data suggests that conversions are likely to exist in most crossover tracts. Negative crossover interference and weak chromatid interference were observed at the population level. These findings aid in the understanding of meiotic recombination and have implications for both basic and applied research.

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P17

eQTL analysis to discover functional regulatory variation

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To find functional variants from among tens of millions of SNPs in maize I am using association mapping (GWAS) to determine the genetic variation that controls tens of thousands of intermediate expression phenotypes. Because the majority of natural phenotypic diversity is controlled by altering expression patterns instead of changing the coding sequence of genes, these expression quantitative loci, or eQTL, are likely to point to true functional variants. These functional intergenic SNPs often go uncharacterized in genetic screens because their phenotypes are more nuanced than knockout mutations. However, by using expression values as quantitative traits in association studies, eQTL can be found on a genomic scale. Using a previously published set of 25.8 billion RNAseq reads (Fu et al 2013), a pipeline was developed to align reads and calculate expression values from the immature kernels of 368 diverse maize lines. After determining that the expression phenotypes had a median narrow sense heritability of 0.32, the expression values were used as phenotypes to conduct 39k GWAS experiments. In addition to calculating covariates (PCs) to account for population structure, hidden factors (HFs) were calculated from the matrix of 368 x 39k phenotypes to control for unmeasured sources of confounding. After accounting for the genetic and unmeasured experimental sources of structure using PCs and HFs, tens of thousands of significant cis and trans eQTL were found for thousands of genes. These eQTL point to functional variation in the form of local and distant regulators of expression.

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P18

The Reinvention of MaizeGDB

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MaizeGDB, the USDA-ARS genetics and genomics database, is a highly curated, community-oriented informatics service to researchers focused on the crop plant and model organism Zea mays. MaizeGDB facilitates maize research by curating, integrating, and maintaining a database that serves as the central repository for the maize community. In 2009, the first publicly released reference assembly became available. At this time MaizeGDB became sequence driven while still maintaining traditional maize genetics datasets. The research focus of the maize community has continued to evolve, making it necessary to continually redefine the paradigm for data access and data analysis tools. This poster will highlight the latest reinvention of MaizeGDB to meet maize researcher's needs and facilitate their goals. Our goal at MaizeGDB is to create a redesign that expands the overall functionality of MaizeGDB while simultaneously creating a clean, modern interface with enhanced user interaction and improved response times. The redesign involved creating a new look and feel as well as reorganizing existing data and incorporating new data, data types, and analysis tools (including, e.g., gene models, diversity data, and functional genomics datasets) into the MaizeGDB resource. A key component to the redesign has been community involvement by offering their perspectives via email, website feedback, and personal interactions. Here we provide an overview of the new website, including new features, data types, and services.

Funding acknowledgement: United States Department of Agriculture (USDA)

P19

Stewardship of the Maize B73 Reference Genome Assembly

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An accurate and well-curated reference genome is necessary to advance maize research, but accomplishing this is a challenging and long-term undertaking. To support this effort, tiling path files describing the maize B73 v3 reference assembly have been loaded into the NCBI database developed for Genome Reference Consortium (GRC) assembly management, to enable the tools and procedures developed for GRC curation of the human, mouse, and zebrafish genomes to be applied to maize. The GRC has developed a number of curation tools and standardized procedures for viewing, assessing, and repairing the genome assembly. .It uses an assembly model (PLoS Biol. 2011 Jul;9(7):e1001091) that supports "patch releases" in between major assembly releases that provide corrections and new sequence without changing chromosome coordinates. In addition, an issue tracker has been set up at MaizeGDB to collect issues in the current assembly gene models. Examples of issues are: BAC tiling path problems, evidence to fill or extend gaps, gene models that need to be split or merged.

Before the genome could be loaded into the GRC database, two rounds of corrections were made to the GenBank records for the roughly 16,000 BACs that were used in the assembly to ensure the portion of the BACs used in the assembly matched the B73 v3 pseudomolecule sequences. Here we present information about the stewardship process, tools and views that are provided at MaizeGDB and through our collaboration with NCBI to use GRC tools.

Funding acknowledgement: United States Department of Agriculture (USDA)

P20

MaizeGDB: Using GO for Gene Annotations

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With an increasing amount of information for researchers to search through, ways to facilitate more precise and uniform queries are being developed. The Gene Ontology (GO) project is one such example. The GO project provides a defined set of terms that are used to describe a gene function, gene product, or subcellular location. The Gene Ontology has a hierarchical structure, which enables genes to be annotated at an appropriate level of specificity.

In order to use the ontology, terms have to be assigned to gene models and products. Here we describe the process used at MaizeGDB to provide these associations (gene annotations). This involves manually sifting through the literature and using several outside resources to provide the most accurate annotations for gene functions, products, and subcellular localizations. Then, the referenced literature along with each gene annotation are associated with GO terms. These GO annotations, available at MaizeGDB, enable researchers to use standard and uniform queries to search for information about genes using their functions, products, and subcellular localizations and to find genes with related functions and products through the hierarchical relationships within the GO ontology.

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

P21

Diversity Panel Phenotype Data at MaizeGDB

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A workflow has been developed to import into MaizeGDB published trait data for public maize germplasm that has been evaluated to explore diversity at the genome (SNP) level. The germplasm include 2 mapping populations, suitable for both QTL and GWAS analyses: the Nested Association Mapping Panel (NAM; 4800 inbreds from crosses of B73 as female with 26 different parents; *Buckler E et al 2009 Science 325:714-718*) and the Inter-mated B73 x Mo17 Panel (IBM; 300 inbreds; *Lee M et al 2002 Plant Mol Biol 48:453-462*). Work is in progress for two other diversity panels: the Goodman/Flint-Garcia Diversity Association Panel (300 inbreds; *Flint-Garcia S et al 2005 The Plant J 44:1054-1064*) and the North Central Regional Plant Introduction Station(NCPRIS)Inbred association Panel (2815 inbreds; *Romay MC et al 2013 Genome Biol 14:R55*). All of the trait data are also accessible as supplemental publication files, and/or posted at the panzea.org website, in various formats. Some 47 traits reported in one or more of 8 publications, and evaluated under different locations, and conditions are represented in the datasets. Challenges met in the data integration included resolving nomenclature for the various germplasm, refinement of a species-neutral controlled vocabulary for traits (the Trait Ontology,), extraction of methods, environments and conditions from publications, and contacting authors in a few instances for clarification. The range of mean values will be reported at the poster for each population and trait, along with sources. Data are available for download at MaizeGDB.

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P22

Comprehensive Comparison of MaizeCyc and CornCyc Metabolic Pathway Resources

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How to choose which maize metabolic pathway resource to use? Currently MaizeGDB has two metabolic pathways resources available for B73: MaizeCyc (developed by Gramene) and CornCyc (developed by Plant Metabolic Network), both created in collaboration with MaizeGDB. Computational pathway assignments for both resources were performed using the same software, i.e., Pathway Tools, however, they differ in their method in assigning enzymatic functions, and therefore have varying degrees of prediction accuracy for different enzyme classes. In addition to differences in computational enzymatic and pathway assignments, both resources have different levels of curation, which is primarily performed by the MaizeGDB curators. Because both MaizeCyc 2.2 and CornCyc 4.0 are available for the B73 RefGen_v2 gene models, here we compare both resources to help maize researchers decide which resource to use when studying their genes of interest. We analyze the enzymatic function space for MaizeCyc and CornCyc and show common and unique enzymatic classes in each resource. We also present an analysis of the new version of CornCyc, CornCyc 5.0, which is based on B73 RefGen_v3 gene models, in computational enzymatic and pathway assignments and level of curation.

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P23

Evolutionary genetics of repetitive DNA in maize and teosinte

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The vast majority of genome size variation in plants is due to differences in repetitive sequence, yet we know little about how selection acts on repeats in natural populations. Here, we use low coverage short read sequencing to investigate genome-wide changes in repetitive content of maize landraces and two teosinte subspecies across altitudinal gradients in Mexico and South America.We map sequences against a curated set of reference repeats to discover the proportion of the genome comprised of each repeat and convert the proportion to a basepair value using flow cytometry estimates of genome size.
Our results in maize accord with previous cytological observations of knobs and show that genome size and most repeat abundances decrease with altitude. In contrast, genome size increases with altitude in natural teosinte populations and certain repeats show patterns different from overall genome size trends.
We use these data to test for evidence of selection on repeat sequence abundance using models of phenotypic and genetic covariance among populations. For several categories of repeats, our results show that population structure and genetic drift alone cannot explain observed clinal patterns, suggesting that natural selection is driving changes in their abundance. For example, we see strong evidence of selection against knob repeats in maize at higher altitudes despite their propensity to drive in meiosis and our evidence that knob abundance increases with altitude in teosinte. Overall, we document complex clinal patterns of repeat abundance both within and among taxa, and demonstrate that many of these trends are the direct result of natural selection.

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P24

Examining alternative splicing in maize and other grass genomes

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Alternative splicing (AS) is one mechanism used to increase proteomic diversity and regulate protein abundance. Using maize as the model system we have been examining the relationship between genetics and methylation on AS. Additionally, we are exploring the extent of conserved alternative splicing across the grasses and investigating the effect of whole genome duplication on AS potential. So far we have used RNA-Seq, EST and full length cDNA sequences to identify tissue specific and genotype specific AS in B73 and Mo17 maize and their reciprocal hybrids. We have also used the intermated B73xMo17 recombinant inbred lines (IBM RILs) to identify cis- and trans-acting regulatory variation associated with splicing (sQTL), some of which overlap loci that encode splicing factors. Identification of AS within gene paralogs that define the two subgenomes of maize reveals a bias in the distribution of alternative splicing. Comparing AS in these paralogous gene pairs to AS within their sorghum orthologues provides evidence for AS sub-functionalization between the maize paralogues after duplication. We will discuss our current results for AS in B73, Mo17 and their reciprocal hybrids, the relationship between AS and gene body methylation, and the identification of conserved AS events across the grasses.

P25
(Poster withdrawn from abstract book)

P26
Exploring allelic variation for response to abiotic stress

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Plants exhibit a variety of phenotypic and molecular responses to environmental stress. Many genes have altered levels of transcript abundance in response to abiotic stress. Maize (*Zea mays*) provides a model system to study natural variation and potential allelic regulatory variation driving gene expression differences in response to abiotic stress. To study the frequency and molecular basis for natural variation in responsiveness to abiotic stress within maize, RNA sequencing was performed on 14 day old maize seedlings of inbreds B73, Mo17 and Oh43 as well as F1 hybrids B73xMo17, B73xOh43, and Mo17xOh43 under control, cold, and heat conditions. The analysis of gene expression levels in control and stress conditions identified many examples of stress-responsive expression in each of the inbred lines. Some of the genes show clear stress-responsive expression in some genotypes but no change in expression in other genotypes. The analysis of allele specific transcript abundance in the F1 hybrids was used to assess the contributions of cis- and trans-regulatory variation for stress-responsive expression. There were a number of examples of cis-regulatory control and current analyses are focused on characterization of the haplotype variation for these alleles. Additionally, examples of trans-regulatory variation that influence gene expression responses to abiotic stress are being mapped by analysis of expression responses in recombinant inbred lines (RILs). Maize exhibits a large amount of natural variation of gene expression in response to abiotic stress and understanding the sources of this regulatory variation may lead to improved strategies to improve abiotic stress tolerance in crops.

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P27

Gene by Environment Interaction in the Maize Ionome

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Plant elemental profiles are determined by the genetics of the plant, the environment it is grown in, and the interactions between them. In order to investigate genotype by environment interactions, we analyzed the maize (Zea mays L.) kernel ionomes of intermated B73 x Mo17 (IBM) recombinant inbreds grown in multiple locations. We measured the levels of 21 mineral nutrients in seeds from eight experiments in four locations over five different years using ICP-MS. Quantitative trait locus (QTL) mapping of these element composition data and a set of 4,217 biallelic markers was implemented with the R packages R/QTL and QTLRel. We evaluated several different methods of QTL analysis, settling on a stepwise forward/backward algorithm with a penalized LOD score in R/QTL and a statistical model including covariates and accounting for kinship in QTLRel. In R/QTL, we mapped data from each location separately and on location/year pairwise differences to find QTL-by-environment interactions. With QTLRel, we mapped QTLs using combined data from all growouts and analyzed GxE by comparing results from models with and without environment as a fixed covariate. As a third strategy, we performed a principal components analysis (PCA) on the elemental composition data and then used these principle components in place of the elemental phenotypes for QTL mapping. Analysis of the PCAs identified additional loci affecting the ionome that were not detected in single element scans, suggesting pleiotropic alleles with multi-element effects. The effect size of the environment and G X E demonstrates that the genetic basis of variation in the ionome is contingent upon growth environment and that this regulation occurs on a multi-element level. Weather data and other location-specific variables were evaluated as possible covariates responsible for environmental drivers of ionomic variation.

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P28

Gene mutual information reveals regulatory network of maize embryo and endosperm development

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The study of maize embryo and endosperm has significant agricultural importance, but remains elusive because of a great number of involved genes and their complex interactions. To better understand the genetic control in maize seed development, we need to reveal the dynamic transcriptional regulatory relationships among transcription factors and their target genes quantitatively. Here we report our integrated regulatory network study using genome-wide spatiotemporal transcriptome RNA-Seq data of B73 maize seed development. Gene expression intensities at all stages were normalized and discretized into bins defined by the B-Spline functions. Then we calculated the entropy of each gene according to its respective distribution probabilities within each bin, which is also known as the marginal entropy. For each pair of genes, their joint distribution under the previously defined bins was taken into account to measure the joint entropy. The mutual information between any two genes was defined as the sum of both marginal entropies subtracting their joint entropy, which indicates the mutual dependence between genes. Transcription factors are major regulators for gene expression, thus transmitting more information to target genes than to unrelated genes or information between non-regulatory genes. Greater mutual information usually suggests higher probability of dependence in general. However some indirect relationships can also contribute to mutual information as well. To avoid false positives caused by these indirect relationships in inferred transcriptional network, we employed relative importance of mutual information indicated by z-scores among all potential regulators and targets, premised on the sparse nature of biological networks. We compared the inferred gene regulatory network to known well-studied genes and found potential transcription factors and genes. We further conducted motif analysis within the same target gene groups. Since functional domains are often involved in transcriptional events, we searched the Pfam database for hits in our enriched set of genes. Network motifs were discovered from the number of edges a node connected to, as well as the topological patterns such as hierarchical structure and network hubs. There are 91 transcription factors and 1,167 genes present exclusively in seed development among an overall of 26,105 investigated genes. This work provides an in-depth dynamic view of the complex regulatory network in maize kernel development.

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P29

Genome-wide analysis of alternative splicing in Zea mays: landscape and genetic regulation

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Alternative splicing enhances transcriptome and proteome diversity in all eukaryotes, and is known to play a role in plant tissue identity and stress adaptation. To catalogue new maize transcripts and genetically map loci controlling alternative splicing, we analyzed more than 90 RNA-seq libraries from maize inbred lines B73, Mo17 and IBM Syn10 DH lines (progenies from B73xMo17). Transcript discovery was augmented with publicly available data from 14 different maize tissues at various developmental stages, expanding the maize transcriptome by more than 36,000. The new transcripts greatly increased the diversity of the maize proteome, in some cases coding for entirely different proteins compared to their most similar annotated isoform. The alternative splicing pattern of many genes was also shown to be regulated over the course of both leaf and seed development. Using the Syn10 DH lines, we demonstrate that the majority of genotype-specific alternative splicing can be genetically mapped, with cis-acting QTLs predominating. These results highlight the currently underappreciated role that alternative splicing plays in tissue identity, genotypic variation and heterosis in maize.

P30

Gramene: A resource for comparative plant genomics and pathways

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Gramene (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 and Reactome. Its strength derives from the application of a phylogenetic framework for genome comparison, and integration of genome annotation and functional data using ontologies. The current release (build 44) includes 39 complete reference genomes, with strong representation of monocots and dicots, as well as lower plants. Species added within the last year include cocoa, peach, wild mustard, wild grasses (including five *Oryza* species), an unicellular green algae, and an ancestral flowering shrub, in addition to a new assembly for bread wheat. For each reference genome we incorporate community annotation from primary sources and enrich this information with a series of standardized analyses. These include functional annotation by InterProScan and classification using controlled vocabularies, Gene Ontology (GO) and Plant Ontology (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, with focus on maize, rice, and Arabidopsis. The current release includes the maize HapMap2 and Panzea's 2.7 GBS variation data sets, the latter consisting of 719,472 SNPs typed in 16,718 maize and teosinte lines. The current release also includes new variation data for sorghum and tomato. In addition to genome browsing capabilities, Gramene produces and hosts metabolic pathways databases and visualization tools. The current Plant Reactome release features the premier of maize and 32 other species, which were derived from orthology projections of rice curated pathways. Gramene is supported by an NSF grant (IOS-1127112) and works closely with the EBI-EMBL, the OICR, and the ASPB.

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P31

High throughput TILLING-by-sequencing in grasses

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TILLING (Targeting Induced Local Lesions IN Genomes) is an efficient reverse genetics technique to identify mutations in genes of interest through PCR-based screens of mutagenized populations. To create this platform for grasses, we have developed both genetics resources and an informatics pipeline to detect rare EMS-induced mutations in pools of individuals using high throughput sequencing technology. To demonstrate the utility of this system, we have established an EMS-mutagenized *Brachypodium distachyon* (Bd21-3) population of approximately 5000 M2 families. By integrating TILLING methods with Illumina sequencing of target gene amplicons, we have developed an informatics protocol for efficiently identifying single base pair mutations. To date, we have screened for mutations in six genes of interest. A total of 67 SNPs were detected and 55 SNPs were confirmed through Sanger sequencing. Therefore, our *Brachypodium distachyon* mutant population and the TILLING by sequencing protocol are valuable and should greatly facilitate reverse genetic approaches for gene discovery in this model grass system. We are currently developing a similar platform for *Setaria viridis*, a model C4 grass, and will present methods and informatics tools that can be easily adopted for maize.

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P32

Hybridization between highland and lowland teosinte populations in the Central Plateau and Balsas River Basin of Mexico

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Hybridization is a largely underappreciated force in evolution. Hybridization improves adaptability through new allelic combinations and can facilitate speciation especially when hybrids are isolated from their progenitors. The teosinte subspecies Zea mays ssp. parviglumis (parviglumis) and Zea mays ssp. mexicana (mexicana) diverged recently, yet they both show clear signs of local adaption as well as ongoing hybridization in hybrid zones. Parviglumis inhabits the lowlands and mexicana inhabits the highlands of Mexico with some degree of overlap at middle elevations. Hybrid zones defined by altitudinal gradients are particularly interesting because of the dramatic differences in environmental factors over a small area. We have identified three zones of clustered hybrid populations between parviglumis and mexicana: one in the Central Plateau of Mexico and two in the Balsas River Basin of Mexico. Hybrid populations were identified using the program STRUCTURE with a publicly available SNP dataset including 983 SNPs genotyped in 2,793 individuals. These data are being used to answer two major questions: 1) What is the evolutionary relationship of these hybrids with each other, maize and neighboring teosinte? 2) Are these hybrid zones stable, locally-adapted populations or are they simply a product of ongoing hybridization in a tension zone between parviglumis and mexicana.

P33

Image-Based Precision Phenotyping of Maize Ear Morphology and Kernel Size

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Grain yield in maize is a complex trait that is highly affected by environmental conditions. This complexity leads to lower heritabilities and makes the mapping of quantitative trait loci (QTL) for grain yield relatively difficult. However, grain yield can be separated into component traits, such as kernel number and kernel weight. Finding and characterizing genic regions contributing to these yield component traits could prove to be important for developing higher yielding cultivars. In order to better understand the relationships between kernel number, kernel size, and cob and ear morphology we have implemented a machine vision platform. This platform uses custom algorithms to extract information contained in digital images to quantify maize ear, cob, and kernel phenotypes. Ear, cob, and kernel images were collected using Epson Perfection V700 Photo flatbed scanners. Ear and cob images were processed to generate width profiles and estimate maximum width, average width, and length. Principal components analysis (PCA) of the width profiles was then used to generate quantitative proxies describing ear and cob morphology. A Fourier transform-based analysis of ear images was used to estimate the average height per kernel along the length of the ear. From images of loose kernels, average kernel width and depth were also estimated. To date, more than 26,000 ears have been analyzed with this imaging platform. Genotype to phenotype associations are being explored using genome wide association (GWA) and joint-linkage mapping.

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P34

Improving Our Biological Understanding of RNAseq

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The field of genomics has grown rapidly with the advent of massively parallel sequencing technologies, which has allowed for novel biological insights with regards to genomic, transcriptomic, and epigenomic variation. One widely utilized application of next generation sequencing is transcriptional profiling using RNAseq. Understanding the limitations of a technology is critical to accurate biological interpretations and clear interpretations of RNAseq can be difficult in species with complex genomes. To understand the limitations of accurate profiling of expression levels we performed simulations of RNAseq in several plant species including Arabidopsis, brachypodium, maize, potato, rice, soybean, and tomato. Reads covering annotated gene models were simulated and aligned using various parameters such as allowing unique vs. duplicate read mapping. This allowed for the identification of genes for which the expression levels might be over- or under-estimated due to homology with other sequences. In maize, nearly 20% of genes were greater than 20% away from the expected count values, suggesting the need for careful interpretation of RNAseq data. Further results of the simulation such as properties of genes showing high levels of deviation from expected values will be presented as well as results from real datasets demonstrating potential misinterpretations.

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P35

Investigating the genetic basis of a maize gene coexpression network

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In maize and in other species, some genes' transcript levels respond in similar ways across development and across populations, and these coexpressed genes often contribute to a specific biological process. Here, using previously published data, we identify genes whose transcript levels change together across a segregating population and identify the genetic basis for this pattern. We construct a gene coxpression network using gene correlations from raw and Fourier transformed data, and we parse the network to identify modules of coexpressed genes. We find the expression levels of genes within gene modules vary across inbred lines, and specific chromosomal loci can explain this genetic diversity. Consistent with this discovery, individual genes' trans eQTL are localized in the maize genome, indicating the presence of a master regulator. In summary, a coexpression analysis of RNA-Seq data from individuals within a segregating population provides a powerful approach to identify the genetic control of regulatory networks. These master regulatory loci could be utilized as selection targets.

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P36

Investigating the sequence, transcription, and translation of the maize B chromosome

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Many plant, animal, and fungi species contain supernumerary, or B, chromosomes that exist as non-essential additions to the A genome. Typically, B chromosomes do not exist in all members of a population, vary in copy number between individuals, possess a non-Mendelian drive mechanism, and do not contain genes required for the survival of the host organism. The maize (*Zea mays*) B chromosome undergoes non-disjunction at the second pollen. Although the exact origin of the maize B chromosome is unknown, it is purported to have arisen from the A chromosomes.

Here, we sequenced flow-sorted B chromosomes to produce a draft *de novo* assembly using MaSuRCA, performed RNAseq to investigate B chromosome transcription, and used LC-MS to search for B chromosome-encoded peptides. The maize B chromosome likely emerged as a composite of some A chromosomes with a portion arriving as a more recent addition to the progenitor B chromosome. The gene-prediction programs Augustus and Maker predicted approximately 7000 genes based on the assembled B chromosome scaffolds. Three hundred nineteen of the predicted genes harbored uniquely mapping transcripts in our RNAseq data, and transcription of at least nine loci was confirmed with RT-PCR. Additionally, many transcripts were found to arise from regions of the B chromosome that were not predicted to encode proteins. Our RNAseq data revealed at least forty-one A chromosome loci that may be targeted by B chromosome-encoded small RNAs in *trans*, suggesting that the presence of a B chromosome may have an effect on the A genome.

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P37

Magnitude and causes of allelic differences in maize (*Zea mays*) short-read alignments

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Next generation sequencing allows the detection and quantification of individual alleles within a sample on a genome-wide level. Accurately detecting the presence and abundance of an allelic sequence within a sample is critical for applications in genotyping and allele specific expression. Many studies across species have reported that nucleotide diversity causes reads from some alleles to fail to align to the reference when the default method of aligning reads to a single reference genome and counting aligned reads derived from each allele is used. Here, we simulate RNA-Seq data from two genetically distinct maize inbreds to investigate the extent and causes of read alignment problems. Reads from both inbreds frequently do not align to their homologous sites within the reference genome. All simulated reads from both alleles align at only 18% of polymorphic sites. As expected, the severity of read mismapping correlates with the number of sequence polymorphisms between the read and reference. Unexpectedly, a remarkable proportion (66.9%) of the sites with unequal numbers of mapped reads have no reads aligned from one parent. Reads from the non-reference parent have better alignments to other genomic positions within the reference genome for over 75% of sites where the allele of the non-reference parent failed to align. Two proposed solutions for mapping divergent reads, either masking polymorphic sites or replacing polymorphic sites, reduce the frequency of unequal mapping globally. Nonetheless, read abundances at many sites continue to be misestimated, and the altered references cause some reads that correctly aligned to their source allele in the reference genome to align elsewhere within a modified genome. For RNA-Seq, a promising approach for accurate read mapping of diverse alleles is to map reads to parent specific transcriptomes. The strong similarity between non-homologous regions of the maize genome presents a major challenge for surveying allelic diversity.

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P38

Maize - GO Annotation Methods Evaluation and Review (Maize-GAMER)

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Maize is an important agricultural crop, accounting for 875 million tonnes of the world's grain production (faostat.fao.org). High-throughput omics studies in maize are hampered by the inadequate number of high-confidence functional annotations associated with maize genes. The Gene Ontology (GO) is a structured set of hierarchically related terms that describe molecular functions, biological processes, and cellular localization of gene products. The majority of the GO annotations for maize are derived primarily using high-throughput pipelines (such as Ensembl), and there is a need to evaluate the confidence of existing GO annotations for maize. Moreover, a systematic evaluation of alternative approaches to assign GO terms is lacking. Here we present a pipeline that assigns GO terms to maize gene models using multiple functional annotation methods along with an evaluation of confidence for these assignments. Our pipeline uses three approaches to assign GO terms: BLAST-based methods, functional domain-based methods, and advanced methods (machine learning and statistical approaches). Using a test dataset that contains high-quality manual annotations from MaizeGDB (~750) and reviewed annotations from UniProt (~6500), we evaluated the performance of our pipeline, compared the performance to other existing annotations, and created a designated set of high-confidence functional annotations for maize genes. Review of these annotations by experts will substantially improve the confidence of annotations predicted using the pipeline, so we have created a user-friendly system to leverage crowdsourcing for manual review of the predicted GO annotations. In addition to enabling researchers to review annotations on an ad hoc basis, we also have developed a mechanism to request help from experts directly based on their knowledge of particular genes' function. Here we present the results of the evaluation of genome-wide GO annotation for maize, introduce and describe the crowdsourcing tool, and request your help to improve the quality of functional annotation of maize genes.

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

P39

Metabolite profiling of ozone stress response in *Zea mays*

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Tropospheric ozone (O3) is a damaging air pollutant to crops, and continued exposure to high tropospheric ozone concentrations are projected to reduce potential maize yields throughout this century. Exposure to O3 causes oxidative stress to vegetative and reproductive tissues, and can affect the abundance of transcripts, proteins and metabolites, leading to accelerated senescence and decreased seed yield. To date, few studies have investigated physiological mechanisms of maize responses to elevated [O3], and no metabolite profiling analyses of O3 response have been done in maize or other C4 plants. In this study, maize inbred line B73 was grown at ambient (~40 ppb) and elevated O3 concentrations (100 ppb) in the field at the Soybean Free Air Concentration Enrichment (SoyFACE) facility in Champaign, IL. Maize leaves from each of 4 ambient and 4 elevated O3 rings and multiple locations within each ring were sampled for metabolite profiling, done using GC-MS, LC-MS and NMR at the University of Florida Southeast Center for Integrated Metabolomics. PCA (Principle Component Analysis) and other analysis will be used to identify which metabolites are most responsive to growth in elevated O3. This poster will present the results of the global metabolite profiling of B73 exposed to elevated O3, and results will combined with physiological and molecular studies to better understand the mechanisms of O3 response in maize.

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

P40

Nutrinet : A Network inspired approach to improving Nutrient Use Efficiency (NUE) in crop plants.

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The goal of our NutriNet project is to identify network modules in crops - exploiting Arabidopsis "Network Knowledge" - that are predictive of phenotypic variation and enhance the efficiency of genetic gain in crop species. We use nutrient use efficiency (NUE) of maize as the target trait to develop and implement this approach. This project involves a controlled nitrogen treatment study across Maize and Arabidopsis lines that show a wide variation in nitrogen use efficiency (NUE). We sampled 9 maize hybrids (B73 X 9 diverse inbreds including IHP1 and ILP1 [Illinois High and Low Protein]) and W22, which exhibit NUE phenotypic variation of interest. In parallel, we are sampling 19 Arabidopsis accessions that show wide variation of NUE traits when grown on low vs. high NO3. Arabidopsis seedlings from these 19 accessions will be grown using low vs. high N-supply to measure NUE traits and with 15N as tracer to measure N uptake rates. Our network discovery pipeline, called NutriNet, will build network modules relevant to NUE variation by combining transcriptome measurements from each of these lines with their NUE variation. Our preliminary results suggest that a core N-regulatory network is conserved between Maize and Arabidopsis. Using the larger study we aim to identify reliable network markers for NUE variation and key transcription factors that regulate them. Finally, Arabidopsis transgenic and mutant lines, and Maize RIL populations will be used to validate the "NutriNet" NUE biomarker genes identified from this study. The outcome of this project will result in the identification of network modules predictive of, or involved in NUE in crops. Importantly, the network-oriented approach to plant molecular breeding developed for NUE is an exemplar, and can be applied to any trait or crop of interest.

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P41

Phenotype discovery enabled by a machine vision pipeline for maize seed and seedling traits

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Establishing predictive relationships between seed and seedling traits benefits basic and applied plant biology research. To discover phenotype relationships between successive life-cycle stages, high dimensional data sets were acquired noninvasively by a machine-vision pipeline. Over 7,000 seeds representing diverse maize (*Zea mays*) inbred lines were used including the 27 Nested Association Mapping (NAM) population parents, 422 members of the Wisconsin Diversity Panel, 162 recombinant inbreds from a B73 x NC350 cross, and 13 kernel composition mutants. Individual kernels were weighed, subjected to near infrared reflectance (NIR) spectroscopy, 3D shape analysis, and then germinated to quantify primary root growth rate and gravitropism with automated image analysis. Kernel height, width, and depth correlated with NIR spectral features representing oil, starch, protein, density, and air space. Position on the cob was tracked for NAM population parents, revealing a kernel NIR phenotype related to density of seed set on the cob. Trends in kernel composition relative to the major axis of the cob were found for a subset of genotypes. Statistical modeling showed that a kernel NIR spectrum could predict strong versus weak gravitropism of the subsequently produced primary root. At different times during the three hour gravitropism time course, kernel weight, density, and oil correlated as highly as r =0.23 with root tip angle. This work shows how a longitudinal phenotype pipeline can discover predictive relationships between life-cycle stages and define new quantitative phenotypes.

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P42

Phenotypic analysis of stress responses in grasses using a high throughput phenotyping system and versatile analysis platform

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Large scale, automated phenotyping is an emerging field filling the gap between high throughput sequencing and trait discovery. Here, we demonstrate the development and usage of the Bellwether Phenotyping Facility, PhenoFront data manager and Plant Computer Vision (PlantCV) analysis platform to obtain and analyze large scale phenotyping data in grass systems including rice, maize and Setaria. The Bellwether Phenotyping Facility houses 1,140 plants in a controlled, automated growth environment and captures fluorescence, near infrared, and visible images of individual plants as they pass through imaging chambers via conveyor belts. Upon completion of an experiment, PhenoFront is used to manage and access imaging and watering data that have been obtained. PlantCV is then used to analyze data from the images quantitatively for trait discovery. Simple traits such as plant height were directly analyzed while complex traits in plant architecture such as tiller number were predicted using secondary measurements including height-width ratio and biomass. Here, we compare multiple growth responses in five rice strains harboring different copy numbers of the DNA transposon mPing in the absence and presence of drought stress, and reveal phenotypes that would be difficult to detect via traditional phenotyping methods.

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P43

Phylogenetic analysis of SWEET transporters in angiosperms

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Plants assimilate CO2 into sugars in leaves and must transport the sugars long-distance through the phloem tissues of veins to non-photosynthetic organs to support their growth and development. Sucrose is the principal sugar transported long-distance in many vascular plants. To enter the phloem, sucrose must cross multiple cell membranes. SWEETs are a recently identified family of sugar transporters that facilitate transport of sugars across biological membranes. Different SWEET proteins preferentially transport various sugars, such as glucose, sucrose, and fructose, and are involved in diverse biological processes, including secretion from nectaries, disease resistance, and phloem loading. However, the biological roles of most SWEET genes have only been demonstrated in dicotyledonous plants and rice, and it is not known if these proteins perform similar functions in other monocots. Presently, several SWEET gene phylogenies have been constructed for a few plant species, but they are incomplete for maize and do not contain data for other economically important monocots.

A phylogenetic tree of the SWEET transporter family was generated using fifteen angiosperm genomes. Using a bioinformatic pipeline, SWEET homologs were identified, sequences aligned and curated, and a maximum likelihood method was used to build the phylogeny. Similar to previous studies, the SWEETs from all fifteen species fell into four major clades; however, several branches of the tree received low statistical support. A synteny-based orthology analysis of the SWEETs from the five sequenced grass genomes was performed to determine if the phylogeny was supported.

These studies identify the SWEET homologs that are most closely related to the characterized SWEET genes in rice and *Arabidopsis thaliana*; the functions of these genes are currently being determined in maize and sorghum. Future studies of these SWEET genes will further our knowledge of how sugar is transported in plants, leading to new advances in biomass partitioning.

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P44

Presence-absence variants and estimates genome diversity in a population of wild maize

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Analysis of resequencing data has provided an in-depth look at species-wide variability in maize and teosinte, but we still have little understanding of how evolutionary processes shape diversity within individual populations. In particular, previous efforts have shown that the vast majority of the maize genome is affected by presence-absence or copy number variation, but we do not yet understand how this genomic flux impacts diversity. Here, we investigate within-population processes and the role of presence-absence variation by resequencing the genomes of 20 teosinte from a single natural population near Palmar Chico, Mexico. We scan the genome for evidence of recent positive selection, and identify thousands of presence-absence variants across euchromatic portions of the genome. We show that patterns of diversity in these regions deviate meaningfully from standard population genetic expectations, and argue that population genetic analysis in complex plant genomes must take into account the effect of such polymorphisms on genome-wide patterns of diversity.

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P45

Regulation, natural variation and functional consequences of DNA methylation

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DNA methylation likely plays an important role in regulation of transposons and genes. The large maize genome with a complex organization of genes and transposons provides opportunities to study several aspects of DNA methylation. Whole-genome methylation profiles in maize have been used to study the genetic factors that regulate DNA methylation, natural variation in DNA methylation and functional consequences of DNA methylation. The analysis of DNA methylation in a panel of maize lines carrying loss-of-function alleles for genes expected to play a role in regulating DNA methylation revealed specific roles for certain genes in controlling context-specific DNA methylation. However, none of the single-gene mutants exhibit major perturbations in the methylome and the apparent lethality of double mutants suggests that maize may require DNA methylation for normal growth and development. The DNA methylation profiles for five diverse maize inbreds revealed that while most DNA methylation patterns are conserved there are still thousands of genomic regions with context-specific changes in DNA methylation. These differentially methylated regions (DMRs) likely include several different sub-types based on analysis of context-specificity of the changes, patterns of DNA methylation flanking the regions and location relative to genes and transposons. The analysis of gene expression in the same tissues of the five inbred lines revealed that most of the differences in gene expression are not associated with differential methylation. Only for genes that show major on-off states in gene expression do we observe a significant association and even for these genes we find that only ~20% are associated with altered DNA methylation. Our findings provide a clearer view of the role DNA methylation may play in creating natural diversity for gene expression and phenotype.

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P46

Regulatory divergence of duplicate genes in maize

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Gene duplication exists in nearly all species and can result in coding and regulatory divergence. In maize, whole genome duplication (WGD) resulted in the subgenomes maize1 and maize2, with maize1 the dominant genome. One unanswered question is the regulatory fate of duplicated genes in gene co-expression networks. To address the consequence of gene duplication on regulatory divergence, we first developed a gene co-expression network from RNA-seq data derived from 64 different tissues/stages of the reference inbred-B73. Next, we used the reference B73 sequence to identify three duplication types: WGD, tandem and inserted (non-syntenic) based on maize syntenic gene blocks and related these duplicated genes to the gene co-expression network. Interestingly, the inserted duplicate genes were less likely to be expressed and more likely to be singletons in the co-expression network, while WGD duplicate genes are expression-prone and likely to be connected in co-expression network. Tandem duplicate genes exhibited moderate expression variation compared with WGD and inserted duplicate genes. In addition, maize1 subgenome dominance was also identified in the co-expression networks – more maize1 intragenome co-expression relationships were identified compared to the maize2 duplicated counterpart. Most intriguingly, a prevalence of inter-subgenome co-expression patterns was identified – more inter-subgenome co-expression relationships than intra-subgenome were uncovered. These results will be discussed in the context of the gene dosage hypothesis.

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P47

Repeat Junction Map Analysis of the B73 Maize Genome

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Current genome assembly algorithms mask highly repetitive sequences, which causes underrepresentation of transposable elements (TEs) and other repeats in the resultant assemblies. However, when a repetitive TE inserts into another repetitive TE or any other sequence, the insertion site creates a unique junction sequence. We have used this concept as a tool for recombinational mapping of repeat junction markers (RJMs).. Now, we have designed Repeat Junction Mapper (RJMapper), as a software tool to automatically improve sequence assembly by using RJMs. We find that the majority of maize LTR retrotransposon RJMs are unique in the genome. With the input of a known LTR retrotransposon database, a query dataset that contains unanchored sequences and a reference dataset, RJMapper links sequences by walking through three modules: (1) locating unique LTR RJMs in the query dataset, (2) mapping query sequences to reference sequences using shared unique LTR RJMs, and (3) anchoring unmapped query sequences to reference sequences using LTR RJM pairing information. Testing of this program shows that RJMapper can unambiguously map >70% of previously unanchored BACs to the B73 maize genome assembly.

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P48

RNA-Seq and ecological niche analysis of a drought-resistant Mexican landrace

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Yield loss due to drought damage is a constraint to maize agricultural productivity worldwide. The Mexican landrace Michoacán 21 (M21) appears to exhibit drought-tolerant phenotypes and may be a valuable genetic resource for breeding varieties for drought-prone regions. However, the underlying genetic mechanisms for drought tolerance in this landrace are not fully understood. In order to explore the potential drought tolerance of M21 we have modeled its ecological niche in Mexico and conducted comparative transcriptomic analyses of RNA-Seq data from M21, B73 (drought-susceptible) and Sorghum (drought-tolerant) under control and drought conditions. To better understand which climatic variables are most responsible for the distribution of M21 in Mexico, bioclimatic GIS data will be paired with latitude and longitude data of sampled individuals of M21 and other (less drought-resistant) landraces. Based on boosted regression trees and the maximum entropy algorithm calculated using the dismo package for R, we will identify climatic variables that best distinguish the geographic range of M21. Identified variables will be inferred to constrain M21's range. In addition to understanding how climate determines M21 range, we seek to understand how M21 responds to drought. Laboratory populations of M21, B73, an F1 cross of the two, and Tx623 (Sorghum) were subjected to drought conditions, and transcripts were obtained from root and leaf tissue samples taken during drought treatment and after recovery irrigation treatment. Differential expression and co-expression analyses will be conducted to identify drought-related expression patterns that differentiate M21 from B73 and to assess, through comparisons to Sorghum, whether mechanisms of drought tolerance are conserved over evolutionary time.

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P49

The Expanded RNA-seq based Maize Gene Atlas: A focus on root development

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Comprehensive and systematic transcriptome profiling provides valuable insights into biological and developmental processes that occur throughout the life cycle of a plant. We have enhanced our previously published gene atlas for maize to now include 80 distinct replicated samples that have been interrogated using RNA-sequencing. The expanded gene atlas includes 51 of the original array-based gene atlas samples that span maize development, a time-course of 12 stalk and leaf samples post-flowering, and a novel set of 17 samples from both seedling and adult root tissues. The entire dataset contains 4.6 billion mapped reads, with an average biological replicate having ~15 million mapped reads, allowing for detection of genes with lower transcript abundance. In addition to describing global expression trends, insights into the root transcriptome are highlighted, as this group of tissues represents a significant addition to the array-based atlas. Remarkable expression differences across a longitudinal gradient of roots were supported by fourfold differential expression of 9,353 genes across four zones of the primary root. Likewise, substantial differences across a transverse gradient of the root were evident from differential expression of 4,728 genes between the cortical parenchyma and the stele of the primary root, two spatially adjacent but functionally diverse root tissues. This comprehensive transcriptome dataset provides a powerful tool and resource for understanding maize development, physiology, and phenotypic diversity.

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P50

The Grass Tribe *Paniceae* and C_4 Photosynthetic Evolution

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Most plants convert sunlight into chemical energy using C_3 photosynthesis. However, a modified pathway, C_4 photosynthesis, allows some plants to be more drought tolerant and fertilizer-use efficient. Strikingly, C_4 photosynthesis has evolved independently in dozens of different plant lineages, a testament to its diversity and advantages in many common terrestrial environments. Currently, massive international efforts are focused on breeding and bioengineering C_4 photosynthesis into C_3 rice and other food and sustainable energy crops. What these efforts often overlook is that there is no "one" C_4 photosynthesis. In fact, with its 60-plus distinct evolutionary origins, 20-plus anatomies, and 3 basic enzymatic sub-types, C_4 is more of a diverse syndrome than it is one generalized photosynthesis type. Because the various C_4 sub-types have evolved in diverse environments, different sub-types may be more efficient for different agricultural applications.

Our study uses transcriptomes from the grass tribe *Paniceae* to investigate the evolution and diversity of C_4 photosynthesis within a phylogenomic context. The *Paniceae* are ideal for this investigation because they are the only plant lineage with representatives that primarily utilize each of the C_4 enzymatic subtypes. This allows us to answer questions such as: How are different types of C_4 photosynthesis related to each other? Which C_3 and C_4 species are ideal model organisms for studying C_4 evolution in the *Paniceae*? Which tribe *Paniceae* species are best suited for development as sustainable energy, food, health, and forage crops?

P51

The iPlant Collaborative: Cyberinfrastructure for enabling data to discovery

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Biology datasets (sequencing, imaging, geospatial, etc.) often exceed the abilities of researchers to share and analyze. Constantly changing software and technologies compound challenges that concern not only to users, but core facilities (genomics, informatics, etc.) who must scale to meet these needs. Managing the lifecycle of data necessitates interdisciplinary collaborations and team science approaches that span multiple departments, institutes, and even continents.
The iPlant Collaborative, a National Science Foundation (NSF) funded cyberinfrastructure project addresses these challenges. iPlant's comprehensive platform is an open source CI. Challenges associated with computational scalability, usability, and extensibility have been addressed by adopting technologies and practices from other science domains experienced with them.
Web-accessible tools and well described application interfaces for data analysis and management of data-driven collaborations leverage federated data and consumption of resources from multiple providers such as NSF funded XSEDE (eXtreme Science and Engineering Discovery Environment), campus clusters, and commercial clouds. Researchers can securely manage and share their data, software tools, and analysis pipelines with their collaborators and/or a large community of users without provisioning their own underlying computational infrastructure. Infrastructure and tutorials are being developed to ease the validation of analysis methods and provide easy access to gold standard and simulation.
Complex analyses are facilitated by using software tools optimized for various high performance and high throughput execution platforms. Web-based Application Programming Interfaces (APIs) support automation and integration of tools and services in other applications and third-party platforms. Learning materials (asynchronous tutorials, webcasts forums, onsite workshops) support all levels of user experience. iPlant's CI provides a gateway to regional, national, and commercial CI, and our toolkits and roadmaps allow researchers to manage data and computation in conjunction with distributed and federated resources.

Funding acknowledgement: National Science Foundation (NSF)

P52

The Maize Genome Project, an Update

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A complete and accurate reference genome is imperative for sustained progress in understanding the genetic basis of trait variation and crop improvement in maize. Although the current B73 reference sequence has seen incremental improvements in quality over the last several years, many gaps and misassemblies remain due to technical limitations in sequence technology relative to the complexity of the maize genome. To remedy this, we are employing PacBio long read sequencing technology to re-scaffold contigs and to fill gaps in the reference assembly. Currently, we have about 17X coverage of the B73 genome. The N50 of the reads in this data set is nearly 15kb, with 43.5% of reads longer than 10kb. We measured the accuracy of the long reads at about 91% by aligning the reads to the current reference sequence. We performed a test run of our gap-filling pipeline with this data set. Our preliminary results showed that the number of contigs in the current pseudomolecules reduced from 137,920 to 80,590. A total of 42.5% of the gaps were filled and 15.8% were extended. The total size of the assembly increased from 2.07Gb to 2.31Gb. To correct sequencing errors, a consensus of the long reads was applied in these newly added sequences. The average size of the closed gaps was 1,434bp. In these newly added regions totaling 265Mb, 77% of bases were identified as repetitive, implying that the remaining 58Mb contains genes or other functional elements missing from the current assembly. With these encouraging preliminary results, we are generating another 20X coverage of long reads to further improve the assembly. At the same time, full-length cDNA sequencing using PacBio Iso-Seq technology is also underway. With the new full-length cDNAs, we are expecting to make the maize B73 gene annotation more accurate, especially regarding alternative splicing. More details of this update will be presented at the meeting.

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

P53

The path of assimilate delivery to developing maize kernels: Contrasting transcriptomes of the maternal phloem-unloading zone and the basal endosperm transfer cell layer

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A primary point of maternal control over nutrient delivery to developing maize kernels lies in the maternal phloem-unloading zone. This zone is immediately adjacent to a second, better-known transfer layer comprised of filial rather than maternal cells (the basal endosperm transfer layer [BETL]). Both of these two transfer layers are critical for normal kernel development. Past work has emphasized the contrast in gene expression between the BETL and other endosperm cells that store starch, but the maternal phloem-unloading zone has received relatively little attention. We therefore compared changes in transcript profiles of the maternal phloem-unloading zone with those of the filial BETL, each cryo-dissected from kernels at 8, 14 and 20 days after pollination (DAP). A total of 6643 differentially-expressed genes were identified (representing ~40% of all genes expressed in these tissues). Of these with contrasting mRNA distributions, about half predominated in the BETL, and half in the maternal phloem-unloading zone. A number of genes were essentially "specific" to either the BETL or the phloem-unloading zone, but most showed gradations of distribution. Functional groupings of mRNAs in both transfer layers indicated that although identities of specific genes differed, there were prominent similarities in putative overall roles (eg. abundant transporters, membrane constituents, and extra-cellular proteins). Transcripts for amino acid transport and N-metabolism were especially abundant in the phloem-unloading zone. Profiles for both portions of the transport path were strongly enriched for mRNAs of pathogen-responsive genes at all stages of development tested, reflective of their dual roles in protection. Genes for abiotic-stress responses were also abundantly represented in both profiles, although those indicative of hypoxia were more evident in the phloem unloading zone. Data indicate distinctive, transport and stress-related functions in the maternal zone of phloem unloading as well as that of the BETL.

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P54

The PlantSEED resource for functional annotation and metabolic modeling of plant genomes, and the generation of tissue-specific metabolic models.

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The past two decades have seen much progress in development of in silico metabolic reconstructions that enable exploration of the relationship between genotype and phenotype. The progress has had three axes: in the number of sequenced genomes, in the annotation of enzymes and reactions, and in better procedures to propagate annotations and construct metabolic models. These axes come together in the ModelSEED, a subsystems-based framework for the automated reconstruction of genome-scale metabolic models of microbes. Building on this progress in microbes, the PlantSEED, which targets plants, has been developed in a collaboration between teams at Argonne and University of Florida. PlantSEED http://plantseed.theseed.org is a specialized niche within the ModelSEED ecosystem geared towards utilizing the plant-specific databases to create an intensively curated set of primary metabolic pathways that can be used to construct plant metabolic models.

There is also growth in –omics datasets, which can be used to refine metabolic models and hence to better replicate metabolic phenotypes and to generate more reliable hypotheses. Here we highlight the importance of the PlantSEED approach to limit the gene-reaction associations to those supported by experimental evidence in order to create a robust genome-scale metabolic model for maize. We utilize various sources of protein localization data to assign reactions to subcellular compartments, and transcriptomics data to filter the reactions that would be active in particular tissues. We highlight the differences in pathways and biomass between maize leaf, endosperm, and embryo-specific metabolic models. PlantSEED can be used to apply the same approaches to other plants.

The genome-scale metabolic models and data used in this work are also available in the DOE Systems Biology Knowledgebase (http://kbase.us).

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P55

The teosinte (Zea mays ssp. parviglumis) *de novo* genome assembly and annotation

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Maize (*Zea mays* ssp. *mays*) was domesticated from the wild grass teosinte (*Zea mays* ssp. *parviglumis*) approximately 9,000 years ago in the Balsas River Basin of southwest Mexico. Insights regarding gene loss, gene gain, and large-scale structural evolution during domestication can be discovered by comparing the genomes of teosinte and maize. Characterization of these phenomena will improve our understanding of the domesticated phenotypes plant breeders fine-tune to improve maize production. However, to compare maize and teosinte, high-quality teosinte genome assemblies must first be generated. In this project, we have leveraged preliminary sequence data to assemble draft genomes for two teosinte inbred lines (TIL01 and TIL11). We used three different de novo assemblers (RAY, ALLPATHS-LG and MaSuRCA) on XSEDE High Performance Computing clusters to generate the draft assembly. Our preliminary results indicate that MaSuRCA has performed better in terms of total genome assembly length, CEGMA genes coverage and other assembly metrics. The assembled scaffolds for TIL01 (1.5 gigabases) and TIL11 (1.2 gigabases) cover approximately 50% of the estimated teosinte genome size. Annotation of these genomes are being performed using Maker-P software. Our plan is to use additional long reads (PacBio) and RNA-seq data to improve this assembly.

P56

Understanding Maize Structural Variation Via BioNano Genome Mapping

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Genomic structural variation (SV), comprising unbalanced forms that are copy number variation and balanced forms that are the sequences in same copy number but at different genomic locations (e.g., inversions and translocations), has been implicated in phenotypic traits. Maize exhibits high levels of SV. However, SV, especially balanced SV, has not been well explored in maize. The Irys genome mapping technology from BioNano Genomics provides direct visualization of long, single DNA molecules (100-500 kbp). We have used this technology to generate >100x depth of Mo17 genome mapping data. The *de novo* assembly resulted in a physical map (BNG CMAP) that consists of 1,212 contigs and approximately 2.18 Gbp. A comparison between BNG CMAP and the *in silicon* CMAP based on the B73 reference genome discovered extensive differences between two maps. The differences are presumptively largely due to SV between B73 and Mo17. Our pilot results demonstrated that BioNano genome mapping is a useful tool to understand maize SV.

Funding acknowledgement: Kansas State University startup fund

P57

Utility to the Maize Community of a Functional Gene Discovery Platform for Sorghum Improvement

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Even though we sequence and resequence genomes on a regular basis, the functions of most genes still remain unclear, limiting the utility of these genome sequences for plant breeding. Mutations that alter gene function and phenotype remain the most powerful tools in understanding what genes are doing. The relatedness of maize and sorghum make studies moving back and forth between their genomes particularly powerful as a tool for validating gene function in both crops. We created a population of ~12,000 EMS-induced mutants in sorghum and have resequenced 600 of these mutagenized genomes thus far, identifying ~60,000 homozygous point mutations in protein coding sequences (see the poster by Addo-Quaye et al). **This is a publicly available, sequence-indexed collection** and can be analyzed using both forward and reverse genetic approaches to connect mutation to phenotype and gene function. Genes of interest then become targets for further study in the naturally diverse germplasm that is the core of improving sorghum as a food, feed and fuel crop. To this end, we have also sequenced the genomes of 30 diverse sorghum inbreds and landraces and indexed the variation within each gene for these lines. Genome sequence is available for an additional 44 sorghum lines (Mace et al, 2013), presenting the maize community with a valuable comparative resource. We have focused on mutations within coding sequence thus far, and mutants that show phenotypes in valuable end-use traits. However, mutations in orthologs of genes interesting to the maize community, and mutations we have induced in noncoding sequences conserved between maize and sorghum (and other grasses) are an untapped resource that should be useful in understanding gene function and regulation in both species.

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P58

5-Hydroxynorvaline, an abundant non-protein amino acid in maize

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Non-protein amino acids, often isomers of the standard 20 protein amino acids, have defense-related functions in many plant species. A targeted search for non-protein amino acids in maize identified 5-hydroxynorvaline in the vegetative tissue and seeds. The highest constitutive level of 5-hydroxynorvaline was observed in the brace roots of mature plants. 5-Hydroxynorvaline accumulated to a higher concentration in response to insect feeding and treatment with the plant defense signaling molecules jasmonic acid and salicylic acid. When added to artificial diet at concentrations similar to those found in maize leaves, 5-hydroxynorvaline significantly decreased the growth and reproduction of corn leaf aphids. Drought stress caused even greater increases in 5-hydroxynorvaline content than insect feeding, with the concentration of this amino acid steadily increasing until the plants died. Among the parental lines of the maize nested association mapping (NAM) population, there is a greater than ten-fold range in the constitutive 5-hydroxynorvaline levels. A subset of recombinant inbred lines from the NAM population was used to map quantitative trait loci for 5-hydroxynorvaline accumulation to maize chromosomes 5 and 7.

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P59

An update on bulk segregant analysis mapping of the *carbohydrate partitioning defective* mutants of maize

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Carbohydrate partitioning entails the flux of assimilates from photosynthetic tissues to various sink tissues such as the roots and developing ear. However, an understanding of the genetic regulation of this process remains limited since few of the genes involved have been identified. To elucidate the genetic regulation of carbohydrate partitioning in maize, our lab has been conducting a long-term screen for *carbohydrate partitioning defective (cpd)* mutants showing excessive carbon accumulation in the leaves. As interesting new mutants have been identified, we have backcrossed them into B73 and/or Mo17, and performed bulk segregant analysis (BSA) mapping to provide an initial chromosomal map position. This information is valuable for identifying potential complementation groups if multiple mutants map to the same region, and for ultimately identifying the responsible gene using a map-based cloning approach. Here, we provide an update on new *cpd* mutants and our mapping results.

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P60

Using Bulk Segregant Analysis and Next-Generation Sequencing to Identify Novel Carbon Transport Genes in Maize

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The transport of carbon compounds, especially carbohydrate nutrients, is a critical part of maize development. Sugars are produced in source leaves and then moved to sink tissues and organs by way of a complex network of transport molecules and developmental cues. In order to reveal novel genes that are necessary for the regulation and maintenance of this process, we have identified over 100 putative EMS mutants that exhibit phenotypes consistent with defects in normal carbon partitioning. For ~60 of these families, we have crossed to polymorphic inbred lines either as introgressions or to make F2 mapping populations, and isolated DNA from mutant plants and from nonmutant siblings to create bulked samples for mapping by Bulked Segregant Analysis. These bulks were assessed using Illumina Maize SNP50 microarrays, scoring the allele frequency for each inbred parent at more than 56,000 single nucleotide polymorphisms (SNPs) across the genome. Samples of the inbreds were compared directly to identify informative SNPs in each case (~26,000 for B73 X Mo17, and 19,000 each for B73 X W22 and B73 X Oh43 crosses), typically allowing us to map to within 1-3Mb. Mutants initially described in uncharacterized germplasm were mapped by introgressing into B73 and assessing B73 rare alleles. We are evaluating multiplexed exome sequencing as a way to identify the causative SNPs in the mutants. These alleles represent a valuable, new resource for better understanding the regulation and mechanisms of carbon partitioning.

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P61

A Biochemical Basis for Adult Plant Resistance in the Maize-CCR1 Pathosystem

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Cochliobolus carbonum race 1 (CCR1), the causal agent of Northern leaf spot, is potentially one of the most destructive fungal pathogens of maize. A key virulence factor involved is HC-toxin, a cyclic tetrapeptide with broad-spectrum histone deacetylase activity. To counter HC-toxin, maize has evolved the *Hm1* gene which encodes HC-toxin reductase (HCTR), an NADPH-dependent enzyme that inactivates HC-toxin. Resistance conferred by *Hm1* is highly effective, operating in all parts of the plant at every stage of development. In contrast, resistance provided by *Hm1A*, a naturally-occurring allele of *Hm1*, and *Hm2*, a duplicate gene, is developmentally regulated, becoming fully effective only at maturity. Cloning of these adult plant resistance (APR) genes has revealed that *Hm1A* has five amino acid substitutions, while *Hm2* encodes a truncated enzyme lacking the 52 C-terminal amino acids. Given that their transcriptional and translational levels remain unchanged during development, the APR phenotypes of *Hm1A* and *Hm2* are expected to be dictated post-translationally. We have confirmed weakened HCTR activity in all the APR alleles tested by quantifying the amount of HC-toxin reduced by leaf protein extracts. We have also expressed the HM1, HM1A, and HM2 enzymes *in vitro* and are currently determining their kinetic parameters in part to determine if levels of the NADPH cofactor underlie APR. To further investigate the potential role of NADPH in defining APR, we have quantified temporal and developmental changes of *in planta* leaf NADPH levels.

P62

A maize MADS-box protein is identified to regulate the transcription of zein genes through its interaction with Opaque2

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Zeins are most prominent storage proteins in maize endosperm. Zeins are encoded by multiple genes and gene families, however only two transcriptional factors for zein genes were identified so far (Opaque2 and PBF1). In this study, we identified a new MADS-box transcriptional factor through a yeast-two-hybrid screening against Opaque2. The MADS-box protein can interact with Opaque2 in vitro and in vivo. The N terminal portion of the MADS-box protein contains nuclear localization signal. Interestingly, while the C terminal portion contains transcriptional activation domain, the activity was blocked when full length protein was tested. Transgenic RNAi knockdown lines were generated. Transcriptome analysis of RNAi knockdown lines revealed the down regulation of αzein and 50kD γzein genes. EMSA and mutagenesis assay indicated that the MADS-box protein binds CATGT motifs presented in promoters of these zein genes. The MADS-box alone was not able to transactivate αzein and 50kD γzein promoters. However, when both Opaque2 and the MADS-box protein were presented, the transactivation activities on αzein and 50kD γzein promoters were greatly enhanced. Furthermore, when both AD-truncated O2 and the MADS-box protein were presented, the transactivation activities onαzein and 50kD γzein promoters were still enhanced. Therefore, the interaction of the MADS-box protein with O2 released its blocked transcriptional activition on zein genes.

P63

A maize RWS-GFP haploid inducer line

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Doubled haploid (DH) technology is a useful tool for maize breeding and gene discovery. The production of large numbers of DH lines has been possible recently with the development of various haploid inducer lines based on the discovery of the initial Stock 6 inducer line by Ed Coe. However, problems still exist for the identification of haploid kernels among the diploid majority diploid following crosses by an inducer line. Most of the haploid induction systems make use of the R-nj gene whose expression in the kernel produces diploid kernels with colored aleurone crowns and scutella, and haploid kernels with colored aleurone crowns but colorless scutella. However, the R-nj gene expression depends on various genetic and environmental factors, which may mask the typical R-nj phenotype. To solve this problem, we introduced a dominant GFP marker to a maize haploid inducer, RWS, to produce a RWS-GFP inducer, which allows the identification of haploid in the early germination stage by checking the GFP expression of germinated kernels. Germinated diploid seeds will produce GFP fluorescence in emerged radicles and coleoptiles, but haploids will be GFP negative because of the lack of paternal GFP gene during hybridization with a male haploid inducer. This system has been used in various maize lines successfully, and thus has demonstrated its wide application in maize genetic studies and DH breeding.

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P64

A zebra-band phenotype results from mutation of a PPOX-like gene (*protoporphyrinogen oxidase IX-like*)

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The causal mutation was sought for a zebra-band phenotype of maize leaves in which cells near midribs and major vascular bundles show a distinctive pattern of non-green tissues. Wide, non-green bands form perpendicular to the leaf axis on an apparently diurnal basis. Each of the cross-bands arises from a series of closely-packed, parallel, non-green stripes associated with vascular bundles. The whitest stripes within the cross-bands sometimes include narrow, open gaps where cells have died. The cross-bands appear in the first to fifth leaves, seldom elsewhere, and are most pronounced in field-grown plants. Some aspects of the phenotype resemble other zebra-band mutants in maize, but cross-bands of this one are distinctive in their vascular localization, particular developmental timing, and degree of severity. This mutant was identified among UniformMu seedlings, where it co-segregated with a single Mu insertion in F2 material. The insertion was located in the second exon of a *Protoporphyrinogen oxidase IX (PPOX)-like* gene. The association was confirmed by presence of the same phenotype in a second and third allele from the UniformMu population. Insertions in these additional alleles were located in the second exon and 5'UTR. Although roles of PPOX have been studied extensively in Arabidopsis and elsewhere, the effects of its mutation have not been previously linked to diurnal, zebra-striping. The PPOX enzyme catalyzes the last step in common for both heme and chlorophyll biosynthesis, but if the reaction is defective, then toxic, photosensitive precursors accumulate. Deficiencies in PPOX thus lead to light-induced lesions in organisms from plants to humans. An additional contributor to the diurnal banding observed here in maize is the probable involvement of light- and clock-regulation shown in other organisms for upstream genes in this pathway. Further analysis will determine the relationship between this PPOX mutation and that of the zebra-banding series in maize.

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P65

Allele Specific Responses to Salt and UV in Maize

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In order to understand the allelic variation and hybrid responses for response to salt and UV stress in maize, we extracted and sequenced RNA from 14-day old seeding of inbred lines B73, Mo17, Oh43, as well as the F1 hybrids derived from them, grown in standard conditions (control) and conditions subject to salt (watered with 300 mM NaCl 20 hours prior to collection) or UV (UV-B lamps 2 hours) stress. Each of the inbred and hybrid genotypes exhibited many changes in gene expression in response to each stress treatment. While many of the genes exhibit similar changes across genotypes there were some genes (7%) that exhibit stress-responsive expression in genotypes but not others. Allele-specific expression was analyzed for these genes to understand the contribution of *cis*- and *trans*-regulatory variation to the differences in allelic stress responsiveness. Based on the allelic expression levels in the F1 hybrids we were able to identify examples of both *cis*- and *trans*-regulatory variation. Future work will focus on determining the molecular basis for the regulatory variation. In addition to monitoring allele-specific expression responses in the hybrid plants we also assessed how the differences in per-gene expression levels in response to stress in hybrid plants compared to inbreds. Interestingly, many of the genes that show differences in expression response in the parents tend to have similar responses to the stress as the non-responsive parent in hybrids.

Funding acknowledgement: National Science Foundation (NSF)

P66

Alternative Mutagens for Maize

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The induction of heritable changes in the genome has been an important tool of both basic and applied genetic research in maize as well as other organisms. Mutagenesis provides an effective way for analyzing the function and mode of action of individual genes as well as more complex pathways composed of multiple interacting networks of genes. An allelic series for a locus is extremely powerful when analyzed together to define function; this is especially true where complete loss of gene function results in lethality.The depth and breadth of such an allelic series will depend on mutation spectrum, mutation frequency and the number of families that can be screened.

In maize and many other plant species, EMS (ethyl methanesulfonate) has been the chemical of choice for generating mutagenized populations. However, the mutation spectrum of EMS is limited, with the vast majority of sequenced EMS-induced mutations being G/C to A/T transitions. Thus, only a small fraction of the possible mutational spectrum is explored by EMS mutagenesis. In order to broaden the induced mutation spectrum in maize, a number of alternative mutagens were tested in seeds, including ENU (N-ethyl-N-nitrosourea), sodium azide and gamma-irradiation. An assay based on leaf sectors indicating a change in the phenotype at the oil yellow1 (oy1) locus was utilized to produce sequences for analysis.

P67

Analysis of the maize cytokinin receptor *Zea mays Histidine Kinase 1* function using *Saccharomyces cerevisiae*

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Cytokinins (CK) regulate a diverse assortment of processes in plants, including cellular division, biosynthesis of chloroplasts, and differentiation within root and apical meristems. Response to CK is regulated through a two-component signal transduction system consisting of a receptor and a response regulator. Two-component signaling systems are highly conserved in bacteria, fungi and plants and allow organisms to sense and respond to external and internal stimuli. Our analysis of the semi-dominant, leaf patterning maize mutant *Hairy Sheath Frayed1 (Hsf1)* identified the maize CK receptor *Zea mays Histidine Kinase1 (ZmHK1)* as the underlying gene. The *Hsf1* phenotype is marked by the outgrowth of proximal leaf tissue (sheath, auricle and ligule) in the distal leaf blade, reduced leaf size, and increased leaf pubescence. Missense mutations in the CK binding domain of ZmHK1 increase ligand binding affinity, resulting in CK hypersignaling and giving rise to altered leaf patterning in *Hsf1*. We are using a two-component signaling assay in *Saccharomyces cerevisiae* to understand the relationship between these amino acid changes and altered ZmHK1 activity. We have assayed the three independent *Hsf1* alleles (*Hsf1-1595, Hsf1-1603,* and *Hsf1-AEWL*) using the yeast system and found some signal in the absence of added CK. We are making additional targeted amino acid changes near the CK binding domain in ZmHK1 to determine which residues are critical for ligand recognition, binding and signaling. Our current results will be presented.

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P68

Assessing the potential for epigenetic gain in maize (*Zea mays*) hybrids through novel epiallele specific heterotic interactions

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A significant advancement in crop productivity has been achieved through harnessing yield gains due to heterosis in crop species such as maize (*Zea mays*). Recent evidence presents a role for the epigenetic regulation of gene expression in heterosis, signalling new opportunities to understand and exploit this trait. The formation of novel epialleles in F1 hybrids may be responsible for many of the novel phenotypic characters seen in F1 hybrid plants. We will further investigate this hypothesis and assess the potential for the generation of novel traits in hybrid maize by modification of the epigenetic landscape in maize parental lines. Epigenetic modifications will be achieved by CRISPR/Cas9 targeted mutagenesis of enzymes involved in DNA methylation and chromatin remodelling, followed by epigenomic profiling of the lines. A diallel cross design will be used to investigate the relative performance of F1 hybrid plants generated from epigenetically modified lines, to identify lines with potential for improving abiotic stress tolerance. We will also build materials for the development of epigenetically recombinant inbred lines (epi-RILs). The construction of diverse epi-RILs in an important crop species such as maize is the first step towards comprehensive investigation of the potential of epigenetic plant breeding approaches. All of the biological resources generated in this project, including epigenetic mutants and lines established for epi-RIL production, will be submitted to the Maize Genetics Cooperation stock centre.

Funding acknowledgement: Irish Research Council

P69

Bioinformatical comparison of maize virus genomes

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The remarkable reserves of high and stable maize yields are consisted in its integrating protection from diseases. Through the disturbance of persistent balance of microorganisms in plant agrobiocenoses favorable circumstances for the development of phytopathogenes appear. Maize is amenable to viral diseases. The most widespread viruses affected maize are *Barley stripe mosaic virus (BSMV)*, *Barley yellow dwarf virus (BYDV)* and *Maize dwarf mosaic potyvirus (MDMP)*. All of them belong to family *Potyviridae* and are (+)RNA viruses. Maize viruses provoke depression of assimilation and transpiration functions of plant, reduced growth and dwarf appearance, partial tassel sterility, sharp fall in productivity. In years of mass expansion maize virus diseases cause 30-55% yield losses. Therefore, investigations of virus genomes are actual for elaboration of marker-assisted techniques of tolerant maize genotypes selection. Comparison of *BSMV* and *BYDV* genomes by bioinformatics resource Blastn and alignment of nucleotide sequences allowed identifying 6 patterns of concordance: 2 in comparison of (+) and (–) strands and 4 in comparison of (+) and (+) strands. Identical regions were 11-15 nucleotides long. Identity was absolute but 2 segments which were not identical within two nucleotides up to fragments ends. Comparison of *BYDV* and *MDMP* showed 1 identical region for (+) and (+) strands and 2 – for (+) and (–) strands. Identified concordant fragments were 12-16 nucleotides long, 2 of 3 fragments were absolutely identical. *BSMV* and *MDMP* genomes were identical once in (+) and (+) comparison and once in (+) and (–) comparison absolutely, concordant fragments were 12-13 nucleotides long. Consequently, regions on nucleotide sequences identical for all three maize viruses are absent. Genomes of *BSMV* and *BYDV* have more common identical regions among themselves than with *MDMP* genome. All the fragments denoted as identical were 11-16 nucleotides long, nucleotide gaps were not discovered.

P70

Characterization and fine-mapping of maize *carbohydrate partitioning defective13* mutant

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Carbohydrate partitioning is the process by which plants move sugars from photosynthetic source tissues (such as leaves) to non-photosynthetic sink tissues (such as flowers, roots, and stems). Although this process is extremely important in the development and growth of plants, little is known about the genes and subsequent proteins that are involved in the partitioning of carbon from source to sink tissues. *carbohydrate partitioning defective* (*cpd*) mutants are characterized by their inability to move fixed carbon from their leaves, leading to starch build up. These mutants can be identified in the field by anthocyanin accumulation in the leaves, leaf chlorosis, and sometimes smaller plant stature. One of these mutants, designated *cpd13*, has recurring transverse chlorotic bands on its leaves and accumulates starch and anthocyanin in these regions. Using bulk segregant analysis (BSA) mapping, the *cpd13* mutation was determined to be located on the lower arm of chromosome 2. Through subsequent PCR mapping using both insertion deletion polymorphisms (IDPs) and simple sequence repeats (SSRs) markers, we narrowed the location of the gene to approximately 1.2 cM. We have also identified a second allele of *cpd13*, designated *cpd13-35*, and both mutant lines are being used for characterization and fine-mapping. Identifying and characterizing the gene underlying this mutation will help determine how plants allocate carbohydrate resources.

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P71

Comparative Shotgun Proteomic Analysis of Isogenic Opaque Endosperm Maize Mutants

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One of the most well-characterized opaque endosperm mutants in maize is *opaque2* (*o2*) which has a chalky endosperm with a substantial increase in the essential amino acids, lysine and tryptophan due to a lower abundance of zein proteins. This proteome rebalancing due to improper zein accumulation is also seen in *floury2* (*fl2*), *Defective endosperm B30* (*De*B30*), and *Mucronate* (*Mc*) but with only marginal increases of lysine. Interestingly, other opaques such as *opaque1* (*o1*) and *floury1* (*fl1*) do not have affected zein accumulation or protein body formation which suggests that there are other factors which contribute to kernel texture. We used a label free shotgun proteomic approach on the non-zein fraction of endosperm proteins to investigate factors involved opacity across a diverse set of isogenic opaques in the W64a maize inbred. Using the University of California-Davis Proteomic Core for generation of our raw LC-MS/MS data Scaffold proteomic software was used to identify approximately 7000 proteins experiment-wide. Each opaque mutant ranged from the identification of 3000-4500 proteins present. Through pairwise comparison for each opaque mutant with W64a WT, those proteins having significant fold-changes were kept for analysis. This reduced the working set to approximately 100-200 proteins per mutant. An initial clustering and principle component analysis demonstrates two groups of opaques, those with large global effects on the proteome due to zein accumulation or protein body packaging and/or trafficking defects and o1 which is most similar to WT showing a limited proteomic effect. Further enrichment analysis of the proteomic data and biological implications will be presented.

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P72

Dating Maize Centromere Divergence

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Suppression of genetic recombination in the centromere provides an opportunity to reconstruct phylogenies of these regions in maize and teosinte. Non-recombinant regions were identified from deletion patterns and confirmed using HapMap2 [Chia *et al*. Nature Genetics, 2012] data. Alignments were derived from either HapMap 2 data or using custom Perl scripts to call nucleotides from an independently generated bowtie output. Neighbor-joining trees constructed from these datasets of CEN5 had the same topology with high bootstrap values, although the custom alignment used only 0.5% of the HapMap2 alignment. However, estimated internal branch lengths differed by up to 2-fold longer with the custom alignment. In general, teosinte inbreds were located basal to maize inbreds. Reconstruction of the CEN4 phylogeny using HapMap2 data placed all maize inbreds in a single clade that diverged an estimated 6,500 years ago, indicating a selective sweep in maize of this centromere after domestication.

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P73

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 stress-induced conjugation of small ubiquitin-related modifier (SUMO) to large collection of nuclear proteins involved in a diverse array of chromatin and RNA processing events. Despite the importance of SUMOylation to stress tolerance, little is known about the function(s) of 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 SUMOs, E1, E2 and E3 enzymes involved in the conjugation cascade, and an array of deSUMOylating proteases that reverse the modification. Phylogenetic analysis reveals that most plants express a divergent 'non-canonical' SUMO, which has likely evolved through multiple independent duplication events, along with a more ancient, highly-conserved 'canonical' SUMO with essential cellular functions. Additional SUMO isoforms include a conserved SUMO variant with an elongated, charged N-terminal extension followed by the signature beta-grasp fold, and a monocot-specific DiSUMO-like protein bearing two SUMO-type, beta-grasp folds in tandem. The seven-member E2 gene family subdivides into a conserved Class I group that is constitutively expressed, and a cereal-specific Class II group whose expression is more restricted, suggesting that Class II *Sce1* genes have subfunctionalized. Assays using recombinant enzymes demonstrated the functionality of the maize SUMO machinery *in vitro*, and, like *Arabidopsis*, maize rapidly SUMOylates an array of proteins *in planta* upon heat stress. We have developed transgenic germplasm to identify maize SUMOylation targets by proteomics approaches and have isolated a *UniformMu* insertion line that suppresses stress-induced SUMOylation, which will help dissect the functions of SUMO during stress. Collectively, these studies define the organization of the maize SUMO system and provide a springboard for functional characterizations, especially with respect to its role(s) in stress protection.

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P74

Detailed expression analysis of maize Pstol1 homologs in contrasting genotypes for phosphorus efficiency

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Maize is generally considered to have a high fertility soil requirement, so the development of phosphorus-efficient maize genotypes would be beneficial in low-input agroecosystems and would improve the sustainability of high-input agroecosystems. Plants developed several mechanisms to adapt to low phosphorus (P) conditions, indicating that this is a complex trait. The main mechanism that has been implicated with increased P acquisition efficiency involves changes in root morphology. In this context, Phosphorus-starvation tolerance 1 (Pstol1) was identified as the gene underlying the Pup1 locus, which is responsible for enhanced early root growth, P uptake and grain yield in rice and sorghum. Recently, we performed comprehensive QTL mapping in maize recombinant inbred line population (RIL) in nutrient solution under low-P conditions and pointed out candidate genes as maize homologs (ZmPSTOL1, ZmPSTOL4, e ZmPSTOL6) to the rice PSTOL1 (OsPSTOL1) based on QTL co-localization with root and P efficiency traits. In the present study, we aimed to verify the spatial and temporal gene expression of these maize Pstol1 homologs in two P contrasting maize genotypes (L3 – efficient and L22 – inefficient). First, the temporal expression revealed that all genes start to express, in nutrient solution, at 7 days after germination (DAG) and had their peak of expression at 17 DAG. Expression profile of the candidate genes was assessed in different maize tissues (tassel, leaves, stem, seeds and roots) that were harvest during flowering, revealing that ZmPSTOL1 and ZmPSTOL6 were more expressed in roots and tassel of the inefficient line (L22) while ZmPSTOL4 was more expressed in these same tissues but of the efficient line (L3). We also harvested different root parts (primary, lateral, non-embryonic seminal, embryonic seminal, crown) of L3 and L22 grown in nutrient solution at 17 DAG. These results showed that ZmPSTOL1 and ZmPSTOL6 were more expressed in all root types of L22 line and ZmPSTOL4 was more expressed in L3 primary root, especially at the differentiation zone. Finally we correlated gene expression from contrasting lines with root morphology traits. These results shed a light on the illusive Pstol1 pathway; however, further functional studies are required to comprehend the actual pathway leading to root system modulation by Pstol1.

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P75

Determining Heat Tolerance via Chlorophyll Readings and Electrolyte Leakage

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With global climate change predicted to cause increases in temperatures in many parts of the world, it has become increasingly important to study the effects of heat stress on plants. Excessive heat, along with other abiotic stresses, is known to be detrimental to crop yields which could endanger future food security. We have initiated a project to study heat stress in maize with the eventual goal of developing heat tolerant maize germplasm for the developing world. We have done field trials for the NAM founders in India to determine heat tolerance of the various lines and found B97, Mo17, and CML 322 to be among the most heat tolerant while B73 is most susceptible. Based on a literature search and availability of transposon insertions, we have selected certain maize mutants potentially involved in heat stress tolerance. We have measured chlorophyll readings for these mutants in a growth chamber under heat stress as well as taken electrolyte leakage data. We found that three mutants: lipid transfer protein, carbohydrate transporter, and fatty acid desaturase had consistently lower chlorophyll readings than W22, their wild type counterpart. We also found that the electrolyte leakage data for B97, Mo17, and CML322 matches well with the field phenotypes thus potentially providing an accurate, simple assay to test for heat tolerance.

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P76

Developing perennial maize for sustainable agriculture

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Maize (Zea mays, also called field corn) is the premier crop in the United States. While contributing greatly to the US economy, growing field corn is not without consequence. Soil erosion and nutrient runoff in the US corn belt are currently major problems that maize cultivation contributes to because of its short growing season and the need of perturbation of soil through tilling and sowing. Perennial maize, on the other hand, offers the potential for a longer growing season, and importantly, a more developed root system can access soil nutrients and water more deeply in soil. In our study, a hybrid maize F1 has been created, between Z. diploperennis and Z. mays cv. Rhee Flint. Polymorphic molecular markers have been developed that identify the alternative alleles of four domestication genes namely: id1, gt1, tga1 and tb1. These markers have confirmed that we have the F1 and will be used to track the segregating alleles in the F2 and backcross generations. The F1 displays perennial traits for 3 generations of cut-backs for now and segregation of morphological traits is observed among the F2 and F3 generation. Perennial F2s and F3s will be used for perennialism QTL mapping; F3s that will have all four domestication genes allele from Z. mays cv. Rhee Flint will be selected for further breeding use.

P77

Developing protocols for understanding abiotic stress response in maize

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Plants provide a large contribution to the international community's diet and unfortunately the changing climate is their greatest threat. The demand for maize is high (www.fas.usda.gov), but regrettably there are many obstacles in successfully growing the crops. Abiotic stresses such as heat, drought, high salt content, flooding, cold, and variation in light severely affect corn yields (Mittler 2006). It is thought that stressing seedlings may potentially increase crop ability to combat abiotic stress as adults, resulting in "stress training" (Boyko et al. 2010). In order to further investigate this possibility, a series of subsequent stresses needs to be performed. To date, there are no previously described explicit protocols for determining the best way to stress a plant with the potential for it to recover and be "stress trained". The goal of this experiment was to develop a systematic protocol for testing the ability of maize plants to be "trained" for more efficient response to stress as well as further understand the molecular basis for maize stress response. Utilizing laboratory conditions and equipment such as incubators, plant simulated lamps, sodium chloride water, bins and paper clips, cold room, and ultraviolet lamps we were able to experiment with stress. Protocols for six different stress conditions, including drought, flooding, high salinity, cold, ultraviolet exposure, and heat were developed. In addition, it was pertinent to determine what stage of the plant life cycle is the most useful for stress training. Another objective was to determine the duration of the stressor that stressed the plant severely but allowed for recovery and subsequent stress to be administered. The twenty four hour chilling stress as well as overnight chilling for eight hours showed statistically significant difference indicating chilling stress prevents germination and severely affects plant growth and development. Freezing stress even for a very short time resulted in plant death. Flooding also demonstrated statistically significant effects on plant growth and development. RNA samples for three inbred lines and four hybrid maize lines subjected to six stresses were collected and sequenced to investigate response of maize seedlings to stress. Genes responsible for stress response were discovered and characterized. The protocols developed in this project can now be utilized to further discern the ability of plants to be "trained" for more efficient stress response.

Funding acknowledgement: National Science Foundation (NSF)

P78

Dissecting putative roles of maize *Pra1* and *Ndpk1* in C-partitioning and energy balance

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Resource-partitioning between respiration, carbohydrate biosynthesis, and even signaling pathways, depends on ATP/ADP ratios and their balance with other nucleotides (UTP, GTP, and CTP). This balance is mediated by Nucleoside diphosphate kinases (NDPKs), which use ATP to phosphorylate UDP, GDP, or CDP, producing the corresponding nucleoside triphosphate (NTP). To test hypotheses for functional significance of NDPK's in maize, we began with a molecular dissection of *Ndpk1* expression at the mRNA level. Public databases assign eight different mRNAs to maize *Ndpk1*. The three most 5' sequences encode a predicted prenylated Rab acceptor (PRA-1), whereas the five mRNAs further downstream encode NDPK1. Only 900bp separate the *Pra1* and *Ndpk1* sequences. The RNA-seq data from different databases and diverse tissues indicate transcripts arise from two distinct genes. Additionally, alternate splicing leads to at least three different *Pra1* mRNAs, and five from *Ndpk1*. A quantitative appraisal of these eight transcripts by RNAseq and qPCR identified those most abundant in root tips of 7-d-old wild-type seedlings, and in different tissues of 72-day-old plants. The *Pra1* mRNAs were maximal in growing tissues, whereas those of *Ndpk1* peaked in highly-lignified organs (eg. prop-roots). Root-tip data were compared to those from a mutant line in which a Mu transposon was inserted in the Pra1 portion of the sequence. Levels of all *Ndpk1* mRNAs were reduced by 80% in seedlings homozygous for this mutation. Enzyme activity was also reduced by 57%. Results indicate that a Mu-insertion in *Pra1* down-regulates expression of the downstream *Ndpk1*. We have yet to determine the basis for this relationship. However, results thus far indicate that the diverse transcripts from these closely-positioned genes involve differential expression, alternate splicing, and share at least some regulatory features. Collectively these affect functional contributions by *Pra1* and *Ndpk1* at the enzyme and signaling level in each tissue.

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P79

(Poster withdrawn from abstract book)

P80

Dissecting the C_4 Carbon Concentrating Sub-pathway in Maize

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Some of the world's most productive crops, including maize, sorghum, and sugarcane, utilize the C_4 photosynthetic pathway to efficiently incorporate atmospheric carbon dioxide into carbohydrates. C_4 plants exhibit better nitrogen and water use efficiencies, enabling them to produce higher yields than their C_3 counterparts including rice, wheat, and barley. Because of the need to produce sufficient quantities of food for a population expected to reach more than nine billion by 2050, and to reduce the environmental impact of agriculture, there is an increased interest in engineering C_4 photosynthetic traits into C_3 crops. To engineer this complex trait into C_3 crops requires a deeper understanding of the fundamental regulatory networks that underlie developmental, physiological, and biochemical innovations.

To define the networks underlying C_4 biochemistry in maize, an NADP-ME C_4 subtype, several C_4 genes have been characterized using a genetics approach. Interestingly, unlike other NADP-ME type plants, maize also expresses the genes necessary to perform the phosphoenolpyruvate carboxykinase (PEPCK)-type C_4 photosynthesis; however, it is unclear the precise role this subpathway plays in carbon utilization. To address the role of the PEPCK subpathway in maize, we have employed the *Ac/Ds* transposon tagging system to generate stable genetic knockouts in the C_4 PEPCK gene. In screens of nine hundred individuals, eight independent alleles were identified. Initial characterization of homozygous loss-of-function alleles in PEPCK, grown in the greenhouse under high and low nitrogen conditions, result in the accumulation of less biomass, particularly under low nitrogen; flowering is not delayed compared to wildtype plants. We are also characterizing the regulatory networks of the PEPCK subpathway in transgenic *Setaria viridis*. Additional biochemical and physiological experiments will help to further characterize the role of the PEPCK subtype in carbon and nitrogen assimilation.

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P81

Dissecting the molecular genetic basis of shade avoidance response in higher plants: from model species to crops

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Increasing the planting densities has been used as an effective approach for increasing maize yield per unit land area. However, plants compete with neighboring vegetation for light when planting at high densities. The shade of nearby vegetation reduces the ratio of red to far-red light, which causes the plants to trigger a series of responses known collectively as shade avoidance syndrome (SAS): including accelerated elongation of stems, elevated leaf angles to the horizontal, reduced branching and early flowering. Although the SAS is thought to provide an adaptive advantage by increasing a plant's ability to compete for limited resources in natural settings, it is often accompanied by reduced investments in other organs such as roots and leaf blades, and other potentially deleterious effects on a plant's fitness, disease resistance, and/or yield.
Over the past few decades, much has been learned about the genetic networks regulating SAS in the model dicotyledenous plant Arabidopsis thaliana. It has been shown that in Arabidopsis, SAS is mainly controlled by the red/far-red photoreceptors-phytochromes. In addition, a number of positive regulators and negative regulators of SAS have been identified and their signaling mechanisms elucidated. We are taking a multidisciplinary approach to dissect the molecular genetic basis of SAS in maize. Questions we are interested in addressing include: 1. What are the roles of phyA and phyB in mediating SAS in maize? 2. What are the key signaling components acting downstream of phytochromes to mediate SAS in maize? 3. How have the phytochrome signaling pathways been shaped during maize domestication and postdomestication breeding? By answering these questions, we hope to bring a better understanding of the molecular mechanisms regulating SAS in maize, which should facilitate the breeding of shade-tolerant maize by attenuation or refinement of SAS in maize.

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P82

Ds mutagenesis and characterization of multiple carbonic anhydrase genes in *Zea mays*

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Photosynthesis is a vital biological process that supports life on Earth by converting light energy into organic compounds that can be used for food and fuel. To meet the demands of the world's growing population, advancements need to be made to increase efficiency and sustainability of major crop plants. The carbon concentrating mechanism utilized in C_4 photosynthesis affects photosynthetic efficiency and water use efficiency by balancing the exchange of CO_2 and water across the leaf surface. Regulation of this trade-off becomes even more important under stress conditions. Carbonic anhydrase (CA) catalyzes the first dedicated step in the carbon concentrating mechanism of C_4 photosynthesis, the hydration of CO_2 into bicarbonate. To characterize the role of CA in maize, we initiated a directed mutagenesis of three tandemly arranged CA gene copies. The transposable element *Ds* was used to generate a total of 40 insertions in the CA genes, which created single, double and triple mutants. Previously we reported that the requirement for CA is minimal under controlled grow chamber conditions. However, a field grown maize study found the CA locus to be associated with nitrogen metabolism and photosynthesis (Buckler Lab). Thus, a detailed physiological characterization of the mutants was performed in the greenhouse, which revealed that CA plays a role in stomatal response and water use efficiency. In addition, mutant lines were grown in multiple locations to assess the impact of reduced CA under field conditions. These results can be compared to grow chamber and greenhouse experiments to understand at how CA can enable plants to respond when challenged with changing conditions in the field. Taken together these data will enable us to understand the role of CA in maize and potential avenues for optimizing water use efficiency and photosynthesis by altering the carbon concentrating mechanism and stomata.

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P83

Dynamic spatio-temporal distribution of non-structural carbohydrates in corn plants (*Zea mays*) during the reproductive stage

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Starch metabolism plays a central role in the development of plants because it controls **sink-source relationships** among heterotrophic and photosynthetic cells. Previous results have suggested that the vegetative stem of maize has an active turnover of non-structural carbohydrates (**NSC**), since the levels of hexoses and intermediary starch are modified when organs such leaves (source tissue) and cob (sink tissue) are removed. We therefore quantified the spatio-temporal distribution of starch, glucose, fructose and sucrose in **twelve different reproductive and vegetative structures of maize** (node 1, node 2, node 3, internode 1, internode 2, internode 3, leaves, husk, stigmata, shank, cob and grains). Sweet yellow corn was harvested **through five reproductive stages** (R1, R2, R3, R4 and R6). Statistical analysis of variance, linear correlation and maximum information coefficient allowed establishing functional relationships among metabolites and organs. Based on the spatial and temporal concentration of NSC we propose three regulatory points for the flow of carbohydrates from green leaves to grains. Results are presented using a custom figure format employing grayscale imaging to represent metabolite levels numerically throughout the plant. Besides the seed endosperm, other maize structures can store large amounts of intermediary starch (node 2 attached to the shank of the female inflorescence, shank and cob). A progressive accumulation of starch was observed in stigma from stage R2 to R6. The metabolic patterns suggest distinct functions for each maize structure during the reproductive phase. Overall, a very active and dynamic metabolism of NSC was demonstrated in the vegetative and floral stem of maize during development prior to pollination or seed maturation. Such information will help us better understand the complex and dynamic process that controls cob growth, silk elongation, carbon translocation, harvest index, photosynthesis and final grain yield.

Funding acknowledgement: Centro de Investigación y de Estudios Avanzados del IPN-Unidad Irapuato.

P84

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 (emb) 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. However, the phenotypes of plastid translation mutants are highly genetic background dependent. When plastid-translation-related emb mutants isolated from the W22 inbred UniformMu maize population were crossed to the B73 inbred and subsequently self-pollinated, suppression of the emb phenotype was observed in F2 seeds, conditioning viable embryos that germinate to produce albino seedlings. To test the hypothesis that background suppressors specifically modify plastid translation mutants, we analyzed a set of emb mutants from the UniformMu transposon population that included genes involved in plastid translation as well as essential genes that have non-plastid functions. We generated F2 populations of these emb mutants as described above and found that the genetic background suppression of emb phenotype was exclusive to plastid translation-related mutants. We then mapped the genetic suppressors using SNP markers. Two suppressors were identified located on the short arm of chromosome 5 and long arm of chromosome 10, respectively. Based upon the mapping results and the segregation ratios of albino seedling in F2 populations, a two-gene model is proposed: mutant plants that are homozygous recessive for either of two suppressor loci produce albino seedlings. In addition, we observed that the two suppressors modified leaf width of albino seedling in a dosage- and allele- dependent manner. Fine mapping of the two suppressor loci is under way with the goal of identifying candidate genes. Our genetic and molecular analysis of embryo lethal suppressors will provide insight into the specific role of plastids in embryogenesis.

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P85

Endosperm Carbohydrates During Kernel Development in Pseudostarchy and Extreme-sugary Maize (*Zea mays* L.) Inbreds

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The process of starch formation in pseudostarchy *(su1-ref/su1-ref)* endosperm in which isoamylase1, a debranching enzyme, is not active, is not clearly understood. In wild type maize, isoamylase1 is required for the production of amylopectin. By evaluating mature and immature kernel tissues of pseudostarchy, sugary, and wild type inbreds, we can determine polysaccharide concentrations during endosperm development as well as determine if an increase in amylose is responsible for the accumulation of starch in pseudostarchy inbreds. After seven cycles of divergent selection from a base population fixed for the *su1-ref* allele, three pseudostarchy inbreds and four extreme-sugary inbreds were selected. The seven inbreds plus Ia453 *Su1* and Ia453 *su1-ref* were planted in 2013 and 2014 in a randomized complete block with two replications. At 14, 21, 28, 35, and 50 (maturity) days after pollination starch and water-soluble polysaccharides (WSP) concentrations were measured. Sugars, amylose, and amylopectin concentrations were measured at maturity. Pseudostarchy inbreds did not significantly differ from Ia453 *Su1*. Wpse2 and Wpse3 contained starch and WSP concentrations similar to Ia453 *Su1*, and had less sugar than the extreme-sugary inbreds and Ia453 *su1-ref*. Amylose and amylopectin ratios of Wpse inbreds did not differ from Ia453 *Su1* indicating that the accumulation of starch is not due to an increase in amylose. These results imply that an unkown debranching enzyme is unregulated in pseudostarchy lines allowing the formation of normal amylopectin.

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P86

Enhanced sulfur assimilation drives expression of the sulfur-rich seed storage proteins in maize

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Sulfate reduction from sulfate to sulfite catalyzed by the 5'-adenylylsulfate reductase (APR) serves as the major regulatory point in the sulfate assimilation pathway. Bacterial APRs can functionally substitute for the more complex plant APRs and expression of these bacterial enzymes in Arabidopsis and maize are able to enhance sulfate assimilation. However, constitutive expression of bacterial APR in maize appears to interfere with normal development. Therefore, we used the tissue-specific *RbcS* and *PepC* promoters to test whether expression of bacterial APRs in bundle sheath or mesophyll cells would be an improved method for sulfur (S) assimilation in maize and increase the levels of the S-containing seed storage proteins, which are part of the zein multigene family, in the seeds. Four expression constructs (P_{RbcS}-EcPAPR; P_{PepC}-EcPAPR; P_{RbcS}-PaAPR; P_{PepC}-PaAPR (Ec - *Escherichia coli*, Pa - *Pseudomonas aeruginosa*)) were tested for their utility in enhancing sulfate assimilation in maize seeds. Transgenic plants were generated that showed increased methionine-rich 10-kDa δ-zein and the cysteine-rich 16-kDa β-zein. The maize APR mediates a sulfate assimilation pathway that is more akin to that of PaAPR's than EcPAPR's. Surprisingly, we found that EcPAPR has a more pronounced effect on S-rich zein accumulation than PaAPR. Several backcrosses of the transgenic events to the B73, B101 (BSSS53), Mo17, and A654 inbred lines exhibit differing patterns of S-rich zein accumulation, reflecting position and background effects. Indeed, one transgenic event expressing the P_{PepC}-driven EcPAPR results in an 18- and seven-fold increase of the 10-kDa δ-zein in a B73 and B101 background, respectively, relative to the null transgenic plant. This integration event would be a sufficient dominant marker to eliminate synthetic methionine supplementation in animal feed.

P87

Evidence for maternal control of seed weight in the Krug Seed Size selection population and derived lines

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Maize is an important cereal grain used as livestock feed, staple food and biofuel. Seed size and seed weight are related yield components which are important in maize domestication and artificial selection. Therefore, a 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 contribute to this goal, inbred lines derived from two maize populations, Krug Large Seed (KLS30) and Krug Small Seed (KSS30), were developed to investigate mechanisms controlling seed weight differences in these populations. Transcriptional and phenotypic measurements were take on the inbred lines and their reciprocal hybrids. Phenotypic measurement of seed weight, seed length and width revealed that KLS30 inbreds have a range from 167% to 277% greater seed weight as compared to KSS30 inbreds. Analysis of reciprocal hybrids relative to their inbred parents indicated that there was a significant effect of the maternal parent for seed weight and for seed size. In addition, imaging of developing kernel sections by microscopy showed that the large seed size of KLS30 inbreds is largely explained by increased cell number rather than cell size. Comparative transcriptional profiling of parental inbreds and the corresponding hybrids at two developmental stages was surveyed using RNA-seq. Differential gene expression between the small-seed group and large-seed group revealed a variety of molecular functions and biological processes with the most notably enriched in nutrient reservoir activity, carbohydrate metabolic processes and response to stimulus. Specifically, sucrose metabolism, glucose synthesis and plant hormones categories including GA and JA biosynthesis pathways were the most enriched categories. Hierarchical clustering of transcriptional profiles showed clustering of the reciprocal F1 hybrids and their respective maternal parent, consistent with a maternal contribution to seed weight. Weighted gene coexpression network analysis identified several eigengene modules, modules associated with seed weight were enriched with genes involved in specific metabolic and developmental processes such as gluconeogenesis, GA inactivation and seed development which were consistent with the differentially expressed gene analysis. These results expand our understanding on the molecular mechanisms controlling seed size and weight in maize.

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P88

Exploiting Maize Leaf Development to Identify Networks Underlying C4 Differentiation

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Maize (Zea mays) is one of the most important food, feed and fuel crops in the world and is also one of the most productive. Like many of the world's most productive plants, maize utilizes C4 photosynthesis to fix carbon using two anatomically and biochemically distinct cell types. Two features of C4 plants are close vein spacing and a photosynthetically active inner bundle sheath (BS) with large, numerous starch-filled plastids that are arranged centrifugally around the cell. In contrast, mesophyll cell plastids are arranged randomly in the cell and do not accumulate starch. The maize leaf is an ideal system to study C4 differentiation as it is subdivided into two organs, a low vein density sheath and a high vein density blade. The lg1 mutant blurs the normally distinct boundary between sheath and blade tissues and provides an opportunity to follow the differentiation process by extending the transition from C3-like to C4 tissue. Several hormones have been implicated in the control of vein patterning, a key C4 trait, but little is known of the regulatory network that controls this differentiation process. Here I describe our preliminary data utilizing RNAseq analysis to define the network of genes associated with C3 and C4 tissues at the boundary of blade and sheath in wild type and the lg1 mutant.

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P89

Fine Mapping and Characterization of Genes Involved in Nitrogen Utilization Efficiency within Maize

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Nitrogen Utilization Efficiency (NUtE) is an important component of yield determination within agronomic crops. However, high levels of nitrogen application on crop production areas can have negative effects on both the environment and human health. Despite its importance, relatively little is known about the genetic mechanisms that govern NUtE within inbred or hybrid maize varieties. Previous work has utilized a genetic mapping study of a hybrid population developed from the intermated B73 X Mo17 recombinant inbred line (IBMRIL), test crossed to the Illinois high protein 1 (IHP1) inbred line, which has altered N utilization. We identified 10 robust QTL associated with NUtE that range in size from 14-9030 kbp and aim to identify causal genetic variants. Publically available gene expression data for the IBMRIL has been used to help narrow down potential candidate genes within these QTL that likely contribute to differences in NUtE. In addition, analysis of allelic divergence between a diverse set of Stiff Stalk and Non-Stiff Stalk inbreds, which is indicative of past phenotypic selection for high grain yield, has provided another means of prioritizing the most promising candidate genes. Currently, we are developing genetic markers to fine map the genomic region of interest. Once identified, the effects of these genes on NUtE will be verified in the field using various resources such as near isogenic lines (NILs), mutant lines and transgenic plants. The results of this project could be used to develop maize varieties that require lower nitrogen inputs and therefore would reduce costs for farmers and mitigate environmental and health effects associated with high ambient nitrogen levels.

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P90

Fine-mapping and characterization of *carbohydrate partitioning defective47* mutant

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Photosynthesis is the process in which plants convert carbon dioxide to sugars using energy from the sun. Typically, photosynthesis occurs in source organs such as leaves, the fixed carbon is then transported through an intercellular vascular network called the phloem, and then the photoassimilates are stored in sink organs such as seeds and roots. This transport and allocation process is called carbohydrate partitioning. A group of mutants in maize are unable to adequately transport sugars and they are designated as *carbohydrate partitioning defective* (*cpd*) mutants. We have identified a *cpd* mutant called *cpd47* that is characterized by stunted growth and displays anthocyanin accumulation on most of the leaf blades, especially towards the tip. The latter phenotype is associated with the hyper-accumulation of starch in the leaves. Currently, we are using fine-mapping to locate the *cpd47* gene using Insertion/Deletion (InDel) and microsatellite PCR-based molecular markers. In addition, we have identified a second allele of *cpd47*, called *cpd47-28* that we are also characterizing to confirm and better understand the function of this gene. Results from this work will be valuable to manipulate genes underlying carbohydrate partitioning in maize for better yield production.

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P91

Functional consequences of evolutionary changes of CENH3 in maize

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The centromere specific histone H3 variant, CENP-A, also known as CENH3, has an important role in proper chromosome segregation in cell division. Although the function of the centromere is conserved in eukaryotes, the CENH3 protein sequence is highly diverged among species. The sequence of centromeric satellite repeats is also highly different among species. One hypothesis to explain these rapid changes is coevolution, where divergence of centromeric satellite repeats requires change in the protein sequence. We investigate this hypothesis by testing whether exogenous *CENH3* can complement a *cenh3* mutation in maize. We describe the maize *cenh3* mu-insertion mutant as well as preliminary results of complementation using *CENH3* from *Sorghum*, which is closely related to maize but has a distinct centromere repeat, and *Oryza*, which despite its greater phylogenetic distance shares some repeat homology with maize.

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P92

Functionalization and use of novel nanomaterials in chromatographic separations and imaging

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The development of new stationary phases and supports, such as functionalized monoliths or structured thin films, for high-performance liquid chromatography (HPLC) has led to improvements in the performance and efficiency of many types of separations. The combination of HPLC with a separation technique in which biologically-related ligands are used as binding agents is referred to as high-performance affinity chromatography (HPAC). This method makes use of the specificity of these binding agents and the high-resolution and speed of HPLC. One type of support material that has been used to make microcolumns, microchips, and capillaries for HPAC is a monolith. Monoliths can be based on either inorganic (silica) or organic (polymeric) materials. The porosity of a monolith allows for quick analysese and low backpressures to be achieved in separations. The combination of monoliths with a biologically related compound is referred to as affinity monolith chromatography. Structured thin films (STFs) have been used to perform ultra-thin layer chromatography (UTLC) and can be prepared from monolithic materials or through the process of glancing angle deposition (GLAD). UTLC has been recently used to rapidly separate small molecules such as lipophilc dyes and sugars. Two long-range goals of this current project are 1) to prepare monolith supports containing immobilized proteins (e.g., antibodies or transport proteins) for use in HPAC and 2) to analyze the properties of structured thin films that are comprised of silica or alumina through the use of ellipsometry and UTLC. The development of these supports and microcolumns are expected to yield faster and improved separation methods for chemical analysis and in the combination of these separations with on-line imaging.

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P93

Gametophytic incompatibility in maize: Refining the region of interest

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Gametophytic cross-incompatibility has recently attracted much attention for its value in maintaining purity in organic maize fields in close proximity to genetically modified maize. Several gametophytic incompatibility systems in maize have been identified, Ga1-s, Ga2-s, and Tcb1-s; yet, current understanding of how these systems function at the genetic level is still lacking. Each system is controlled by a gene (or cluster of genes). Our research seeks to utilize the Ga1-s system to better understand its function in maize. This locus is responsible for cross incompatibility between maize varieties and could serve as a genetic barrier to prevent adventitious presence. Three naturally occurring varieties of ga1 have been identified: ga1 (lacking a pistil barrier and the ability to pollinate females with the pistil barrier), Ga1-s (possessing a pistil barrier to ga1 pollen and having the ability to pollinate pistils with the barrier), and Ga1-m (lacking the pistil barrier but having the ability to pollinate pistils with the barrier). Recombination studies by several laboratory groups have identified a region of interest on the short arm of Chromosome 4 that encompasses Ga1-s. The Ga1-m haplotype was incorporated into a W22 inbred and a bacterial artificial chromosome (BAC) library was developed; four BACs were identified and sequenced. The resulting reads were assembled using the B73 genome sequence as a scaffold. Reads that failed to map to the B73 genome were subjected to de novo assembly. Assembled sequence data reveals many differences between the sequenced variety and the B73 maize reference genome. One or more of these differences likely explain the cross incompatibility response.

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P94

Genetic control of 3-Deoxyanthocyanidins in maize

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In sorghum leaves, *yellow seed1* (*y1*) regulates accumulation of 3-deoxyanthocyanidins (3-DAs) that act as phytoalexins against the fungus *Colletotrichum sublineolum* (Ibraheem et al., 2010). The genetic and metabolic pathway of induction of 3-DAs in sorghum is not very well understood. In maize *pericarp color1* (*p1*), an orthologue of sorghum *y1*, activates the transcription of the flavonoid pathway genes such as chalcone synthase (*chs*), chalcone isomerase (*chi*), dihydroflavonol reductase (*dfr*). In the silks of these maize lines, low levels of luteolinidin (a 3-DA) have been reported. *C*-glycosyl flavones such as maysin and apimaysin that act as insecticidal compounds against corn earworm were also detected in these maize lines. We took a genetic approach and developed transgenic maize lines carrying the sorghum *y1* gene. Two independent maize transgenic events *Y1-rr* (red pericarp, red cob glumes) and *Y1-pr* (patterned pericarp, red cob glumes) were selected and analyzed for their interaction with *Colletotrichum graminicola* (Ibraheem et al. 2015, submitted). These results demonstrated that *Y1* induces flavonoid defense compounds in maize leaves making the plant more resistant to foliar pathogens. In order to further learn about the downstream structural genes required for 3-DA biosynthesis, we focused on the chalcone synthase (*chs*) and anthocyanin synthase (*a2*) genes and introgressed their corresponding maize mutants with transgenic *Y1* events. Thus new combinations included *Y1-rr;C2, Y1-rr;c2, −;C2, −;c2* and *Y1-rr;A2, Y1-rr;a2, −;A2, −;a2* and similar combinations carrying *Y1-pr*. We assayed the accumulation of 3-DAs and *C*-glycosyl flavones in silks. Tissues were extracted in acidified methanol and compounds were quantified using reverse phase HPLC. Results of 3-DA and *C*-glycosyl flavone profiling from silks of families carrying different genetic combinations will be presented.

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P95

Got Starch? Decoding the *Carbohydrate partitioning defective4* mutant in maize

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Photoassimilate partitioning is the process by which fixed carbon produced via photosynthesis in source leaves is transported to non-photosynthetic sink tissues (e.g. seeds). This process, also known as carbohydrate partitioning, is fundamental to plant growth and development but little is known about the genes underlying its regulation. We are using a forward genetic approach to identify the genes controlling carbohydrate partitioning in maize (*Zea mays*) by analyzing *carbohydrate partitioning defective* (*cpd*) mutants. First, we screen mutants in the field for phenotypic indications of excess carbon buildup in the leaves (e.g. leaf chlorosis and/or anthocyanin accumulation), then we starch-stain the leaves in the lab to confirm the hyper-accumulation of carbon in the leaves. Once the mutant is confirmed to be a true *cpd*, it is then used in subsequent mapping efforts to identify the gene responsible. One of these *cpd* mutants, designated *Cpd4*, is a semi-dominant mutant phenotypically identified in the field by yellow striping across the leaf blade associated with excess starch accumulation. We are using a map-based cloning approach to identify and understand the function of the normal *cpd4* gene. Using polymorphic markers, we are narrowing the genomic interval containing the *cpd4* gene. By examining the DNA sequence in this region, we will identify candidate genes and sequence them to determine which one is responsible for the *Cpd4* mutant phenotype. Once the *cpd4* gene is identified, it can be characterized to determine its exact role in the process of carbohydrate partitioning in maize.

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P96

Heavy metal genes involved in maize domestication

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Genetic, genomic and molecular evidence suggests that maize was domesticated from Balsas teosinte (*Zea mays spp. parviglumis*) through a single domestication event that initiated in Central Mexico close to 9,000 BP. Although the developmental mechanisms that gave rise to this phenotypic transformation are under close scrutiny from a human selection perspective, little is known about the possible environmental factors that influenced this evolutionary process. The elucidation of the Palomero Toluqueño genome indicated that at least three genes involved in heavy metal transport and root homeostasis show molecular evidence of being affected by artificial selection, suggesting a role during domestication. The analysis of their genetic diversity on 23 maize landraces and 17 accessions of *Zea mays spp. parviglumis* suggests that all three show reduced genetic diversity across large segments of their coding sequence, resulting in a loss of nucleotide variability as compared to Balsas teosinte. DNA extracted from ancient samples (aDNA) found in San Marcos cave (5,000 - 5,300 Cal BP) allowed multiple library construction for next-generation sequencing technologies, and recovery of several aDNA genomic segments mapping to portions of each of these three genes and corresponding to specific allelic variants, allowing a comparison of nucleotide variability in ancient and extant maize samples. We expect that a detail analysis of their structure, function and evolution will reveal the potential that abiotic stress played in the origins of maize.

P97

Identification and characterization of candidate genes involved in chilling responses in maize *(Zea mays L.)*

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Chilling stress can have severe impacts on the growth, development and productivity of maize. In this study, cDNA-AFLP analysis was used to evaluate gene expression in maize during chilling treatments (6 ℃) over four time periods (0, 2, 6 and 12 h). A total of 441 transcript-derived fragments (TDFs) induced by low temperature treatment were detected. Based on the sequence analysis, the 58 TDFs of known functions were involved in metabolism, photosynthesis, signal transduction and defense responses etc.The full-length cDNA, encoding CLC-D (chloride channel D) was isolated from maize through in silico cloning and named as *ZmCLC-d*.In plant cells, anion channels and transporters are essential for key functions. Members of the chloride channel (CLC) family located in intracellular organelles are required for anion accumulation, pH adjustment, and salt tolerance. Here, we cloned a maize CLC gene, named *ZmCLC-d*, and found that its transcription was up-regulated under cold, drought, salt, and heat stresses, and after hydrogen peroxide (H_2O_2) and abscisic acid (ABA) treatments. The overexpression of *ZmCLC-d* in *Arabidopsis* conferred tolerance to cold, drought and salt stresses; this tolerance was primarily displayed by an increased germination rate, root length, plant survival rate, antioxidant enzyme (catalase, peroxidase, and superoxide dismutase) activities, and a reduced accumulation of Cl^- in transgenic plants as compared with wild type (WT) plants. The accumulation of H_2O_2 and superoxide anion in leaves of the *ZmCLC-d*-overexpressing plants was much less than that of the WT plants. The expressions of some stress related genes, such as *CBF1*, *CBF2*, *CBF3*, *DREB2A*, and *RCI2A*, increased to a greater extent in the *ZmCLC-d*-overexpressing plants than in the WT. Our results strongly suggest that *ZmCLC-d* played an important role in stress tolerance. This study provides important clues to understanding low-temperature regulation mechanisms in maize.

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P98

Identification of four QTLs for herbivore-induced terpene production in maize

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Terpenes are secondary metabolites with many important roles including growth regulation, plant defense and communication with other organisms. After herbivory, maize plants emit a mixture of terpenes. These terpenes attract natural enemies of the herbivore, thus reducing damage to the maize plant. Our aim is to identify the signal transduction pathways that control the emission of these herbivore-induced volatiles.

To identify regulatory factors of herbivore-induced terpene production, a Nested Association Mapping population of 5000 recombinant inbred lines, derived from 26 parent lines, was created. These lines were screened for herbivore-induced volatile emission. From this data, Quantitative Trait Loci (QTL) for terpene emission were calculated. Such QTLs may correspond to either a structural enzyme or a regulatory element that influences the production of one or more terpenes. We identified four QTLs that each affect a different subset of volatile sesquiterpenes:

QTL_{215} for Bergamotene, Farnesene, TMTT, Nerolidol, DMNT, and Linalool;

$QTL_{88\text{-}89}$ for Bergamotene, DMNT, Linalool, and Caryophyllene;

$QTL_{991\text{-}996}$ for Bergamotene, Farnesene, TMTT, and Nerolidol;

$QTL_{404\text{-}406}$ for Bergamotene, Farnesene and Nerolidol.

These QTL regions do not encode enzymes of the terpene biosynthesis pathway, but appear to contain regulatory elements. Since each of the QTLs only affects a subset of compounds, we assume that there are at least three different regulatory pathways. We want to identify the regulatory elements associated with the QTLs. Candidate genes in these regions are investigated for their function in signal transduction. Herbivore-induced expression of such candidate genes provides an additional hint that the gene has a role in the signaling cascade.

Funding acknowledgement: Deutsche Forschungsgemeinschaft (DFG)

P99

Identification of regulatory elements for the production of herbivore-induced terpene defenses.

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Plant secondary metabolites can serve as plant defensive compounds or mediators of chemical communication. Maize plants attacked by caterpillars release a mixture of mono- and sesquiterpenes. These can either have signal properties, defend their plants against herbivores, or attract enemies of the herbivores. In our effort to study the molecular base of these indirect defense mechanisms, we want to identify genes responsible for volatile terpene biosynthesis and its regulation.

About 5000 recombinant inbred lines of a Nested Association Mapping (NAM) population derived from 26 inbred lines were screened for herbivore-induced volatile production. The variation of volatile emission within the NAM population enabled us to identify a set of important quantitative trait loci for volatile terpene production by nested association mapping (NAM). We identified a QTL_{406} on chromosome 3 which is associated with terpene production. This QTL may encode an enzyme of terpene biosynthesis or a regulatory element. Two candidate genes were found at this locus. The first encodes a prenyltransferase (*fpps3*). FPPS activity is crucial for sesquiterpene production and corresponds to the QTL phenotype. Transcript levels in Ky21, the line which strongly contributes to the QTL, are reduced not only for *fpps3* but also for other terpene biosynthesis genes. This and the presence of identical *fpps3* alleles in B73 and Ky21 indicate that *fpps3* is probably not associated with this QTL. The second candidate gene encodes a transcription factor (TF). Sequence comparison of the transcription factor alleles of B73 and Ky21 identified a deletion of 41 amino acids in Ky21 that may inactivate this factor. Transcript levels showed an early upregulation of this TF after herbivory in both maize lines. This suggests a regulatory function of this TF in the regulation of terpene biosynthesis.

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P100

Identification of simple carbohydrates in Corn stalk juice using a linear trap analyzer and DIESI in MS.

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Metabolomics is defined as the characterization, identification, and quantification of metabolites resulting from a wide range of biochemical processes in living systems; being supported by mass spectrometry as one of the valuable methodologies in studies concerning detection and identification of metabolites found in samples of corn stalk, including carbohydrates compounds such as sucrose, glucose and fructose as one of the families present in greater proportion in the stem. Technological advancement representing analyzers on tandem combined with direct electrospray ionization facilitates analytical work aimed at determining the metabolic fingerprints and ions identification. Unlike quadrupole analyzers, wherein the ion flux transmitted from the ionization section to the analyzer is continuous and in each cycle the entire ion packet is sent to the detector, in the linear ion trap is permissible by the technique MSn select an ion of m / z value in particular by successive fragmentations, establishing metabolic patterns for a molecule of interest. Due the physicochemical properties of carbohydrate molecules the reading option is applicable in a negative mode. Fragmentation patterns delivered the following common ions (m/z): 112.92, 178.92, 214.92, 292.80, 376.92, and a cluster formed by the 366.20, 367.12, and 368.04 ions derived from lithium isotopic chemical features. The resulting files were saved as mzXML format using TOPPAS VIEW option of Mass Lynx 4.1 software to analyze and visualize the spectrograms. KEGG-compound metabolites data base was used for peaks identifying. The linear ion trap analyzer allows for high reproducibility, sensitivity, resolution, precision, and it is quantitative, also. In this study we used four different basic ionizing reagents in order to assess their ionization ability: lithium chloride, LiCl which retrieved the best results; ammonium hydroxide, NH4OH; sodium fluoride, NaF and ammonium fluoride, NH4F.

Funding acknowledgement: Conacyt (Consejo Nacional de Ciencia y Tecnología), Cinvestav (Centro de Investigación y de Estudios Avanzados del IPN)

P101

Identifying the gene responsible for *carbohydrate partitioning defective7* mutation of *Zea mays*

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Carbohydrate partitioning is the biological process in which carbohydrates (e.g., sucrose) are transported from photosynthetic source tissues (e.g., mature leaves) to non-photosynthetic sink tissues (e.g., developing leaves, ears, and roots). Although this process is essential for plant growth and development, the regulation of carbohydrate partitioning and the genes involved are not well understood. The *carbohydrate partitioning defective7 (cpd7)* mutant of maize was identified by pale-green coloration of the mature leaves and progressive anthocyanin accumulation within these pale-green regions. Staining these regions for starch revealed hyper-accumulation of starch in *cpd7-1* mutant leaves as compared to leaves from wild-type siblings, indicating the *Cpd7* gene plays a role in carbohydrate partitioning. Two additional mutants with similar phenotypes, *cpd7-2* and *cpd7-3*, were verified to be allelic to *cpd7-1* through complementation testing and mapping. To identify the causative mutation, a positional cloning strategy was undertaken, delimiting a 60,000 bp region on chromosome 9, containing two candidate genes. A complementation test between *cpd7* and a *Mutator* transposable element insertion in one of the two genes has led to the identification of the putative causative gene, which is currently being sequenced in *cpd7-1*, *cpd7-2*, and *cpd7-3*. This information will aid in the identification of genes involved in controlling whole-plant carbohydrate partitioning.

Funding acknowledgement: National Science Foundation (NSF)

P102

Improved DNA extraction from high starch maize tissue using a Sodium Dodecyl Sulfate extraction method

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The standard phenol-chloroform method of nuclear genomic DNA extraction used for maize seedling and leaf tissue is not efficient in obtaining high quality, high molecular weight DNA from starchy tissue (such as corn and wheat seed) and produces yields of approximately 3.19 µg/mg tissue and A260/A280 ratios of 1.4-1.8. However, a Sodium Dodecyl Sulfate (SDS) -based extraction method using a high salt content (0.6M) has proven to be more effective at purifying DNA from the starch in seed tissue than both a prior low-salt SDS and the phenol-chloroform based methods. The high-salt SDS method does have a higher level of degradation when used for seedling tissue, but is able to produce significantly higher yields of high quality genomic DNA from starchy tissue than do the current methods. This high-salt SDS buffer-based DNA extraction method offers an inexpensive, reliable and high-yield method of extraction for maize seed DNA, as well as for other grain seed DNA.

Funding acknowledgement: QUEST Award from Virginia Commonwealth University

P103

In search of transcription factors causing differential starch accumulation between the vegetative and the reproductive stem of maize

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Starch metabolism influences harvest index, yield and food quality in a variety of crops. Starch biosynthesis involves several enzymes in different subcellular compartments but major control is exerted by the reaction catalyzed by ADP-glucose pyrophosphorylase (AGPase). This enzyme is regulated by multiple mechanisms at the posttranslational and transcriptional level. In the genome of maize, there are 7 genes coding for AGPase. Recent investigations in rice have identified transcription factors associated with CO2 assimilation and starch metabolism but their maize orthologues have not yet been studied. In the stem of tropical maize varieties we detected a differential accumulation of sugars and starch in the internodes and nodes during development. There is also a massive difference of starch between the vegetative and reproductive stem (female inflorescence). Stem starch is neither photosynthetic nor corresponds to reserve starch stored in seeds or endosperm. We have named it "intermediary starch" since it occurs within the vasculature connecting source leaves with sink organs. Accumulation of intermediary starch is cell specific and it varies during development. It also responds to environmental cues (water and nitrogen stress) and decreases according to source or sink manipulations. The aim of our project is to document the occurrence of intermediary starch and to identify transcription factors causing its differential accumulation. Our main strategies are: 1) comparative transcriptomic analysis of maize tissues with differential starch accumulation. 2) phenotypic and biochemical analysis of near-isogenic lines (NILs kindly provided by Sherry Flint-Garcia, USDA, Missouri). 3) analysis of the promoter regions of 7 AGPase genes, 4) candidate gene analysis. For that purpose we would like to establish collaborations to obtain seeds of homozygote mutants. During this conference we will show data on metabolite levels and list NILs with altered accumulation of intermediary starch or AGPase activity to pinpoint chromosomal regions of interest.

Funding acknowledgement: Consejo Nacional de Ciencia y Tecnología (CONACyT)

P104

In vivo and *in vitro* analysis of the maize *RNA Binding Motif Protein 48 (RBM48)* splicing factor essential for seed development and plant viability.

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Maize RNA Binding Motif Protein 48 (RBM48) is related to a highly conserved Arginine/Serine-rich (SR) family of proteins that play a key role in splice site selection during pre-mRNA processing. RBM48 is essential for endosperm and embryo development, with the reference allele of *rbm48* disrupting endosperm cell differentiation pathways. Subcellular localization of RBM48 was determined by transiently expressing the full-length maize RBM48 fused with Green Fluorescent Protein (GFP) in *Nicotiana benthamiana*. RBM48-GFP localized to nuclear speckle compartments of the nucleoplasm, which are interchromatin granule clusters enriched in splicing factors. Domain deletion analysis revealed that recruitment of RBM48 to nuclear speckles requires the arginine/serine-rich region. Co-localization and Bimolecular Fluorescence Complementation (BiFC) assays suggest RBM48 interacts with the 65-kDa (U2AF2) and 35-kDa (U2AF1) subunits of the U2 snRNP Auxiliary Factor. U2AF1 and U2AF2 comprise a core splicing factor involved in selection of the 3' splice site of most introns during pre-mRNA splicing. RBM48 and U2AF subunits also co-localize with the ROUGH ENDOSPERM3 (RGH3) protein, which is orthologous to the human ZRSR2 protein. To determine if these co-localization results are indicative of protein-protein interactions, we have raised peptide antibodies against RBM48. Data characterizing the antibodies as well as initial *in vitro* co-immunoprecipitation assays will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P105

Increasing tryptophan and lysine concentration in Zea mays seed.

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Corn and soy are heavily used as the base for livestock feed with approximately 60 percent of U.S. corn designated for this use. Tryptophan is a limiting nutrient for most livestock and is currently added as a supplement produced from expensive industrial microbial fermentation, so corn with increased levels of this essential amino acid is of particular interest to the livestock community. The purpose of this study was to investigate a method of increasing the levels of tryptophan in Zea mays using the *c28* mutant which encodes an alpha-subunit of anthranilate synthase that is resistant to feedback inhibition by high levels of tryptophan. In wild-type plants the feedback inhibition prevents free tryptophan from being present in greater than trace amounts. A method of increasing lysine in the seed, another essential and limiting amino acid, was also investigated, using the *opaque-2* (*o2*) mutation that the production of zein proteins in the enodosperm. The effect of *c28* and *o2*, both singly and in double mutant combinations, on amino acid profiles was characterized using HPLC. Free tryptophan increased significantly from less than 100 parts per million (PPM) typically found in wild-type seeds to greater than 1000 PPM in the *c28* mutant seeds. The *o2* mutation also increased several amino acids, however its effect was greatest on the protein-bound levels of lysine and other amino acids. The significant increase in tryptophan seen in the *c28* mutation was large enough to meet the dietary needs of some livestock; reducing or eliminating the need for supplementing tryptophan to feed.

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P106

Inference of maize population history during migration to highland habitats

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Maize population history is significant for the study of maize evolution. Little is known about the demographical history of maize following its domestication. Colonization in high altitude environments is a major diffusion in maize populations. Highland maize landraces are well adapted to the low-temperature habitats and demonstrated distinct phenotypes, such as denser macro-hairs and greater pigmentation. A founder effect (bottleneck) was found in maize, associated with domestication from their wild relatives. However, the intensity and duration of the bottleneck is controversial. Highland maize landraces are hypothesized to go through an extra bottleneck, as a few individuals diffused to the high altitude and gave rise to currently recognized highland landraces. Here, we sampled four highland populations (the southwestern United States, Guatemala highland, the Mexican Central Plateau and the Andeans) and two lowland populations (the Mexican and South American lowland) for whole genome re-sequencing. It was found that the Andean population has significantly lower genetic diversity compared to lowland populations, but not the other three highland populations. In addition, the MSMC analysis revealed that highland populations exhibited no extra bottleneck, which could be owing to the absence of recovery time from the domestication bottleneck. The Andean population demonstrated a stronger bottleneck compared to all the other populations, consistent with its lowest genetic diversity. In the future, the identified genomic regions, showing evidence of introgression from their sympatric wild relatives, will be masked and the pattern of genetic diversity and effective population size change over time will be re-evaluated.

Funding acknowledgement: United States Department of Agriculture (USDA)

P107

Investigation of Mechanisms Governing Senescence in Maize Using a Systems Approach

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Accumulation and partitioning of carbohydrates (CHO) affects all aspects of grain and biomass yield in grasses and, therefore, strategies to improve yield aim to improve one or both of these processes. Senescence, a highly regulated process involving degradation of cellular components, lies at the interface of both these processes. Senescence is affected by the source-sink dynamics of CHO partitioning and reduces CHO accumulation by disrupting photosynthetic assimilation. We are using a systems approach to identify genetic, molecular, and biochemical mechanisms underlying the regulation of senescence in maize. We have screened of a densely genotyped maize diversity panel (N = 450) and the intermated B73xMo17 (IBM) population (N = 250) for natural senescence and senescence induced by the absence of grain sink. Through ensuing quantitative trait locus and genome-wide association analyses, we have identified candidate genomic regions harboring genetic elements that govern senescence. To complement the genomic approaches, we have generated leaf transcriptome of plants of B73 inbred undergoing premature senescence due to absence of grain sink using RNA-sequencing. Both transcriptomic data, and genetic analyses of isogenic lines derived from B73 and Mo17 were used to further corroborate the candidate genes/regions identified through genomic approaches. We are currently working on cloning and characterizing genes underlying endogenous variation in the onset of senescence. Identification of genetic and molecular mechanisms governing senescence is a key step in devising and implementing strategies to optimize CHO partitioning and enhance CHO accumulation.

Funding acknowledgement: Department of Energy (DOE), Clemson University

P108

Maize as a surrogate for the study of immuno-stimulatory properties of single wheat gluten molecules

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The prolamins are alcohol-soluble storage proteins in cereal endosperms. They have diverged in their structure so that only species in the Triticeae tribe (including barley, wheat and rye) have retained prolamins referred as gluten. In contrast to other prolamins, they can form intermolecular structures that are important for the baking quality of wheat. However, when digested, they can trigger immune response in some humans with HLA DQ2 and DQ8 alleles, a condition known as Celiac disease. Glutenins and gliadins contain tandemly repeated blocks of prolines and glutamines and some of these peptides cause the immune response in Celiac patients. Because of the variation in these tandem repeated motifs, it is sufficient when only one of them is toxic. In addition to the chimerism of these prolamin molecules, they are a mixture of several hundred molecules that cannot be physically separated. We therefore used maize to express single wheat prolamin genes and test their immunostimulatory properties with cultured small intestinal biopsies from Celiac patients. In preliminary experiments, we can show that mutated wheat epitopes retain the capability to cause histopathological changes that are typical of the gluten response.

P109

Maize NAM Founder Lines Differ in Constitutive Resistance to Caterpillar Herbivory

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The maize plant has to face attacks from numerous insects during its life cycle in the field; thus, it needs to employ an array of direct defense responses to protect itself. Direct defense to insects include constitutive and induced defenses. In the current literature, there are very few studies on how NAM founder lines respond to caterpillar herbivory; therefore, this study focuses on assessing resistance to fall armyworm (*S. frugiperda*), one of the major caterpillar pests of maize in the founder lines.

Insect performance bioassays indicted that 26 founder lines show a continuum of constitutive defense to fall armyworm (FAW). Lines in the tropical or semitropical (TS) group possess more resistance to FAW than those in non-Stiff Stalk (NSS) and other genetic groups.

After the initial assessment of resistance, six founder lines, B73 and Mp708 were selected for further study to investigate what contribute to the variation in direct defenses to FAW. Additional bioassays divided them into five susceptible and three resistant lines in regard to constitutive defenses. Next, defense gene expression showed that the ability to mount induced defense to FAW infestation varies among eight maize lines. Also, accumulation of the defensive protein RIP2 and jasmonic acid (JA) were analyzed to substantiate gene expression results.

The results of this study will help to elucidate the natural mechanism of maize resistance to FAW and shed light on the evolution of the maize-caterpillar interaction.

Funding acknowledgement: United States Department of Agriculture (USDA), Intercollegiate Graduate Program in Plant Biology, Department of Plant Science, Pennsylvania State University

P110

Maize Opaque10 (O10) encodes a novel protein body protein that interacts with different zeins

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The maize protein bodies are formed by orderly packing of different zeins. However, the exact process of such packing remained poorly understood. Maize o10 is a classic opaque endosperm mutant with abnormal protein bodies. In this study, O10 was cloned by positional cloning and confirmed by transgenic complementation. It encodes a novel protein only presented in cereals. The O10 protein has several distinct domains. The N-terminal portion of O10 protein can interact with different zeins, such as 19kd, 22kd α-zeins, and 16kd, 50kd γ-zeins. The middle portion of O10 contains a novel seven-repeat domain that is responsible for its dimmerization. The C terminal portion of O10 contains a transmembrane motif that is required for its ER localization. Cellular fractionation assay indicated that O10 is initially synthesized in the cytoplasm, then anchored to the ER, and eventually deposited in the protein body. Lose of O10 function resulted with misshapen protein bodies with altered distribution of zein proteins in o10. These results indicated that O10, as a novel protein body protein, interacts with different zeins and maintains the spherical shape of protein body.

Funding acknowledgement: National Natural Sciences Foundation of China

P111

Maize RNA Binding Motif Protein 48 (RBM48) is Critical to Endosperm and Embryo Development

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SR proteins are a highly conserved family of splicing factors that contain one or more RNA Recognition Motifs (RRM) and an arginine/serine-rich (RS) domain. SR proteins play important roles in splice site selection during pre-mRNA processing. We identified, mapped, and sequenced *Mu* flanking sequence tags (MuFSTs) for *rough endosperm (rgh)* seed mutants in the UniformMu transposon-tagging population. One of these *rgh* mutants co-segregated with a *Mu* insertion in an SR-related protein. Phylogenetic analysis identified the locus as encoding an orthologous human gene annotated as RNA binding motif 48 (RBM48). The human gene is uncharacterized for biological or molecular function in splicing. We identified an independent insertion allele in the UniformMu reverse genetics resource. The *rbm48-umu2* allele co-segregates with a similar rgh phenotype and fails to complement *rbm48-umu1*. These data indicate that the normal *Rbm48* allele is essential to seed development. RT-PCR identified multiple isoforms of *Rbm48* transcripts in roots and shoots of different maize inbred lines. The human ortholog is also alternatively spliced as detected by RT-PCR in cell lines. RT-PCR failed to detect any transcript from the mutant *rbm48-1* allele indicating that the reference allele is likely a null mutant. Prior work has shown that the rgh3 mutant impacts seed development by maintaining endosperm cells in a proliferative state. To test if rbm48 affects endosperm cells similarly, we cultured rbm48 endosperms from a developmental time series. Mutant endosperm tissues were able to proliferate in the latest time point analyzed indicating a similar disruption of cell differentiation as found in *rgh3*. We conclude that RNA splicing has an integral role in promoting endosperm cell differentiation. Future research will focus on the RNA targets of RBM48.

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P112

Maize Transcriptome Regulation In Response To Heat Stress

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Maize (Zea mays) is the most cultivated cereal crop in the world, with an estimated production of 873 million tons in 2013 (FAOSTAT, 2013). Maize is important as a source of biomass for biofuels, raw material for industry and, food for animal and human consumption. It is estimated that food production must double by 2050 to meet the demand of the world's growing population, thus highlighting the importance of further increasing yield and adaptation characteristics. Heat stress plays a major role in reducing yields, especially when coupled with drought. Although numerous genes involved in heat shock or heat stress tolerance have been identified in Arabidopsis, very little is known about the biochemical and physiological mechanisms that contribute to variation in heat tolerance of agronomic crops. To better understand the responses of maize to heat stress, we conducted an analysis of the leaf transcriptome of heat tolerant and susceptible maize genotypes. RNA-seq was used to sequence the entire transcriptome of a heat stress susceptible inbred line (B73) and four tolerant ones (Mo17, B97, CML322, LPS-F32), under optimal and high temperature conditions. Results from these analyses are being used to identify genes that are differentially expressed in response to high-temperature stresses.

Funding acknowledgement: USAID

P113

Mapping the *carbohydrate partitioning defective33* mutant

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Carbohydrate partitioning is the process by which carbon assimilated by photosynthesis is transported from the source tissues of the plant (i.e. leaves) to the sink tissues (e.g. seeds, roots, etc.). It is a vital process in the growth and development of plants but little is known about the genes involved in controlling it. Defects in these genes result in mutant plants incapable of effectively moving the sugars produced by photosynthesis out of their leaves. These mutants are named *carbohydrate partitioning defective* (*cpd*) and can be identified in the field by a number of phenotypes, such as stunted plant height, leaf chlorosis, and/or accumulation of anthocyanin in the leaf blades. One of these mutants, called *cpd33*, is recessive and was found to be allelic to two other *cpd* mutants: *cpd36* and *cpd52*. To identify the *Cpd33* gene, we generated a mapping population, performed bulk segregant analysis (BSA) mapping, and determined that the *Cpd33* gene was located on the lower long arm of chromosome 8. We are currently using insertion deletion polymorphism (IDPs) and simple sequence sepeat markers (SSRs) to fine-map the exact location of *Cpd33*. This work will allow us to better understand the process of carbohydrate partitioning in maize and determine the exact role of *Cpd33* in this process.

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P114

Mechanism of benzoxazinoid exudation by maize roots

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Benzoxazinoids are indole-derived secondary metabolites that are produced in large amounts in young maize plants and protect them against pathogens and herbivores. Benzoxazinoids are also exuded by the roots, potentially to condition the rhizosphere and act as allelopathic agents. Whereas the benzoxazinoid biosynthesis pathway has been elucidated, there is a lack of knowledge regarding the mechanism of exudation. We aim at filling that gap and shedding light on the mechanisms that control benzoxazinoid export from the roots. First, we measured endogenous and exogenous benzoxazinoid concentrations in the parental lines of the nested association mapping (NAM) population. Pronounced differences were found in the relative release rates between B73 and Mo17. We therefore decided to use the IBM 302 mapping population for QTL mapping. In parallel, the maize genome database was screened for potential transporters. The two approaches should provide a short list of strong candidate transporters. Identified candidate transporters will be characterized by expression analysis, heterologous expression and reverse genetics. If successful, the combination of phytochemistry, molecular biology and genetics will result in the identification of the first benzoxazinoid exporters.

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P115

Metabolomics and Climate Change – Antioxidant Enzyme Profile and GC/MS Analysis of Crop Metabolites

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Plant adaptation to stress involves key changes enzymatic processes which shows changes in the expression of genes and gene-products. Our poster examines the physiological and molecular processes for stress adaptation focusing specifically on the antioxidant scavenging enzyme, superoxide dismutase (SOD). Our research examines SOD genes, proteins and metabolite changes after specific crops are exposed to various abiotic stresses (e.g., drought, elevated temperature and salinity). Metabolomics has become a hot topic due to concerns about the effects of climate change on plant resources, biodiversity and global food security. Our work takes both a short-term and long-term look at what role '-omics' research will play in the future development of systematic approaches to addressing how plants tolerate climatic change.

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P116

Multiple roles for 6-phosphogluconate dehydrogenase in maize seed development during heat stress

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Heat stress reduces maize grain weight and quality. Starch synthesis in the endosperm is sensitive to high temperature stress and has the potential to be a limiting pathway for grain yield under heat stress. In addition to enzymes directly involved in starch biosynthesis, chloroplast-localized 6-phosphogluconate dehydrogenase (PGD3) is critical for starch accumulation. PGD3 is one of three enzymes in the Oxidative section of the Pentose Phosphate Pathway (PPP). . Maize encodes two cytosolic versions of 6-phosphogluconate dehydrogenase, PGD1 and PGD2. Homozygous double mutants of pgd1 and pgd2 UniformMu alleles show a variably expressive defective kernel phenotype along with poor plant development. Double mutant seeds phenotypes were analyzed with single-kernel near infrared spectroscopy to predict starch, protein, oil, and density phenotypes. These data suggest that severe double mutant seeds are impacted both in starch and oil accumulation. Surprisingly, PGD1 and PGD2 activity was significantly higher than the reference pgd1 and pgd2 mutant alleles, which were identified in a mixed genetic background and had no impact on seed or plant development. Using the reference pgd1; pgd2 double mutant, we found that the cytosolic PGD1 and PGD2 isozymes are heat stable, while the amyloplast-localized PGD3 is heat labile. These data suggest 6-phosphogluconate dehydrogenase is necessary for starch accumulation in both the plastid and cytosol, but that the PGD3 isozyme may be maladapted for heat stress conditions. To develop a heat stable 6-phosphogluconate dehydrogenase in amyloplasts, we fused the WAXY N-terminal targeting peptide to PGD1 and PGD2 for expression in transgenic maize. These fusion proteins are efficiently imported into pea chloroplasts plastids in vitro indicating that the fusion proteins are likely to be targeted to amyloplasts in starchy endosperm cells.

Funding acknowledgement: United States Department of Agriculture (USDA), Brazilian National Council for Scientific and Technological Development (CNPQ)

P117

New pathways for pathogen induced defenses

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In response to pathogen and insect attack, maize (*Zea mays*) produces a complex series of small molecule defense metabolites. A decade ago, pathogen inducible transcript profiling identified terpene synthase (TPS) genes to be among the most highly elicited in maize. While classical phytoalexins were long considered absent, it is now appreciated that a complex series of both sesquiterpenoid and diterpenoid acids protect against microbial attack. *ZmTps6* and *ZmTps11* both encode β-macrocarpene synthases and are the logical precursors to zealexins. Similarly *ZmAn2* encodes an *ent*-copalyl diphosphate synthase required for kauralexin production. We present recent findings relating to the biosynthesis, structural diversity, and complex biological functions of zealexins and kauralexins in maize. This research also resulted in the discovery of a 3rd unexpected class of pathogen induced metabolites, namely 9-lipoxygenase derived oxylipins that display structural parallels to jasmonates and have roles in direct defense, signaling and cell death.

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

P118

Plastic response to climate change: genetic assimilation in the wild ancestor of maize

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Domesticated crops have long been observed to strongly differ from their wild progenitors in morphological traits. The role of phenotypic plasticity – the ability of an ancestral genotype to express different phenotypes when exposed to various environmental conditions– during domestication is poorly understood. Indeed, domestication occurred during a period of profound global climatic changes. When teosinte, the wild progenitor of maize, is grown in atmospheric CO2 concentrations and temperatures characteristic of the late-glacial and early Holocene periods before and near the time of domestication, it responds with a number of attributes that differ from its characteristics under modern conditions, including maize-like traits in vegetative architecture, inflorescence sexuality, and seed maturation. In this study, we investigate the link between genotype and phenotype in early Holocene conditions to better understand the mechanisms implicated in this plasticity phenomenon. We found a large number of differentially expressed genes, including some thought to be selected during maize domestication. To further elucidate genes involved in the plastic response, co-expression analysis comparing modern day and early Holocene conditions was done using WGCNA. The differential and co-expression analyses point to key genes and pathways that could explain several maize-like traits and lead to a better understanding of maize domestication.

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P119

PRC1 component ZmEMFL1 is required for seed development in maize

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In animals, polycomb group (PcG) proteins form different complexes to repress gene expression by modifying chromatin structure. Polycomb repressive complex 1 (PRC1) and 2 (PRC2) are two well documented PcG complexes. PRC1 catalyzes H2AK119ub1 through an E3 ligase activity of its core subunit Ring1A/1B. Canonical PRC1s contain CBX proteins which can bind to H3K27me3 via the chromodomain, favoring the PRC2-depnedent PRC1 recruitment model. Non-canonical PRC1s, containing RYBP instead of CBX, although cannot recognize H3K27me3, have H2AK119ub1 activity much stronger than canonical PRC1s. In contrast to the evolutionally conserved PRC2 core subunits between plants and animals, PRC1 components are not conserved in plants. Instead, plants have functional counterparts of PRC1 in animals. In *Arabidopsis*, loss of PRC1 leads to gene ectopic expression and loss of organ identity. However, the specific function of PRC1 and PRC2 remains obscure in maize.

We screened a seed-shrinking mutant from B73 EMS mutagenized population. A candidate gene was finally obtained by map-based cloning using a segregated population generated by crossing with Mo17. Sequence analysis of candidate gene revealed a G-to-A transition in the coding region of ZmEMFL1 (EMF-like 1), homology of *Arabidopsis* EMF1(EMBRYONIC FLOWER 1). AtEMF1 is a plant-specific component of PRC1 and is required to maintain vegetative development and repress flower. As in *Arabidopsis*, ZmEMFL1 directly interacted with ZmRING1A/1B in yeast cell. Western blot by antibody specific to ubiquitin showed that H2A ubiquitin level in the mutant was significantly reduced. However, in contrast to *Arabidopsis*, ZmEMFL1 did not interact with ZmLHP1 by Y2H, which recognized and bound H3K27me3 in *Arabidopsis*. Furthermore, the level of H3K27me3 in the mutant was not affected. ZmEMF1 mutant did not show embryonic flower phenotype as in the *Arabidopsis*, indicating unknown different function.

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P120

Progressive Heterosis in Tetraploid Maize

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Tetraploids have several unique characteristics that make them useful for understanding the genetic basis of hybrid vigor. For example, progressive heterosis and inbreeding depression can be markedly higher in tetraploid hybrids than in their diploid counterparts. Here, we examine progressive heterosis, i.e. the additional substantial heterosis seen in 4x double-cross hybrids that is not seen in 2x double-crosses of the same genotype. Using artificially induced tetraploids of B73, Oh43, A188 and H99 and their crosses, we compared single and double-cross hybrids for heterotic phenotypes at the diploid and tetraploid levels in three field replicates. A robust progressive heterosis was found in 4x double-cross hybrids when compared to their 4x single-cross hybrid parents, but this heterosis was not found in the same comparisons at the 2x level.
Based on the complementation model of heterosis, there should be recessive alleles in inbred A that are complemented by B and other recessives in B complemented by A; as well as recessives in C complemented by D and vice versa to account for single-cross heterosis. However, to explain double-cross (or progressive) heterosis, the recessives complemented in A/B cannot match recessives in C/D, otherwise A/B/C/D would have some homozygotes that would lower the heterotic effect. Furthermore, to account for strong progressive heterosis in 4x A/B/C/D, A and B must both contain the same recessives which are not found in either C or D and the reciprocal must also occur with C and D containing similar recessives not found in either A or B.

By analyzing whole genome re-sequencing data from the four inbred lines we do not find the necessary spectrum of alleles to explain tetraploid progressive heterosis under the complementation model. This suggests that some other explanation for heterosis should be sought to accommodate these observations.

P121

Response of the surface lipid metabolome of maize silks to environmental exposure

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The maize silk cuticle provides a primary line of defense between the silk and the environment, and it is thought to play an important role in protection against abiotic and biotic stresses (e.g. drought, UV radiation, insect damage) during the critical period of pollen reception. The silk cuticular surface lipids include long-chain fatty acids, aldehydes and hydrocarbons, and based on their hydrophobic nature they are thought to function as a water barrier. Previous work from our research group has established that surface lipid accumulation on maize silks from the inbred B73 is dynamic. For example, silks that have emerged from the encasing husk leaves exhibit approximately 3-fold more surface lipids than the portions of silks that are still encased by the husk leaves. To further probe the accumulation patterns of these surface lipids in response to the external environment, we have conducted a series of experiments whereby encased B73 maize silks were exposed to the environment one day after silks first emerged from encasing husk leaves. In the first treatment, entire "windows" of husk leaves were excised from a subset of ears to fully expose the encased silks to the external environment. In the second treatment, a series of longitudinal incisions were made in the husk leaves to create a less drastic exposure to the external environment. Ears were harvested two days after treatments were imposed and the surface lipids from both emerged and encased silks were extracted and characterized by gas chromatography-mass spectrometry (GC-MS). Preliminary data from both treatments shows modest increases in concentrations of surface lipids (e.g. long-chain fatty acids) on otherwise encased silks that were exposed to the environment via either treatment. We will discuss the results of these experiments and their implications with respect to the impact of the environment on the composition of the surface lipid metabolome.

Funding acknowledgement: National Science Foundation (NSF), USDA-ARS

P122

Restoring (*E*)-β-Caryophyllene production in a non-producing maize line comprises its resistance against the fungus *Colletotrichum graminicola*

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The sesquiterpene (*E*)-β-caryophyllene is emitted from maize (*Zea mays*) leaves and roots in response to herbivore attack. This compound serves as a signal for the attraction of herbivore enemies and is present in most European maize varieties. However, most North American maize lines have lost the ability to produce (*E*)-β-caryophyllene. Previously we showed that restoring the ability to synthesize (*E*)-β-caryophyllene in a non-producing maize line improved its resistance against the root herbivore *Diabrotica virgifera virgifera*. However, it is largely unknown whether this modification affects the resistance to other pests. In this study we investigated the response of constitutively (*E*)-β-caryophyllene-producing transgenic lines to infection by a hemibiotrophic fungus *Colletotrichum graminicola*. Our results showed that restoring (*E*)-β-caryophyllene synthesis in a Hi-II genetic background enhanced the susceptibility of the plant to *C. graminicola infection* rather than increasing its resistance. This modification did not alter the baseline levels of plant defense hormones and metabolites. Nor did (*E*)-β-caryophyllene production modify the expression of anti-fungal defense genes. Instead, the addition of (*E*)-β-caryophyllene seemed to directly stimulate fungal growth. In an *in vitro* antifungal assay, we found that (*E*)-β-caryophyllene stimulated hyphal growth of *C. graminicola* and *Fusarium graminearum*. Thus although restoring (*E*)-β-caryophyllene production in a non-producing maize line may improve the resistance of the plant against herbivores, it may compromise its resistance to some fungal pathogens. This might explain the loss of (*E*)-β-caryophyllene during maize breeding in environments where *C. graminicola* and *Fusarium graminearum* are prevalent.

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P123

Role of sucrose phosphate phosphatase genes in maize grain filling

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Grain filling is a critical biological process underlying crop yield. To identify key genes regulating grain filling process, we employed Mu-seq, an NGS-sequencing based approach for high-throughput co-segregation analysis of seed mutants. Our analysis identified sucrose phosphate phosphatase1 (*Spp1*) as a key gene in grain filling. SPP catalyzes the final step in the sucrose biosynthesis pathway, hydrolysis of sucrose-6-phosphate into sucrose. The *spp1-umu1* allele, which has a Mutator (Mu) transposon insertion in the first intron of the *Spp1* gene, has a mild shrunken endosperm phenotype that is similar to the classic *shrunken1* phenotype. Maize B73 genome has two Spp genes, *Spp1* and *Spp2*. Expression analyses showed that *Spp1* gene is broadly expressed in maize tissues including embryo and endosperm whereas expression of *Spp2* is typically very low. Consistent with the expression pattern, a Mu induced *spp2* mutant, *spp2-umu1*, had no discernible phenotype, and a seeds of a *spp1 spp2* double mutant were indistinguishable from *spp1-umu1* single mutant. These results indicated that *Spp1* has a predominant role for sucrose synthesis during seed development. We isolated additional alleles of *spp1* mutant by targeted transposon tagging. Whereas several new alleles of *spp1* mutant that conferred mild shrunken phenotype also contained Mu insertions in the first intron of *Spp1* similar to the *spp1-umu1* reference, the *spp1-124* allele which had a partial deletion of the *Spp1* gene could not be made homozygous. Genotyping individual seeds obtained by self-pollination of *spp1-124* heterozygote revealed 1:1 segregation of homozygous wild type and *spp1-124* heterozygous seeds with no homozygous mutant seeds consistent with a block in transmission through one of the gametophytes. Analysis of reciprocal crosses confirmed that the *spp1-124* deletion allele is not transmitted through the male gametophyte. Our results support the hypothesis that re-synthesis of sucrose from imported sugars has an important role in grain development, as well as an unexpected essential function in pollen.

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P124

Sequence comparison of the *starch branching enzyme1* gene among several Native American varieties of maize

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Previously, our laboratory determined that the gene for *starch branching enzyme 1* (*sbe1*) in a high amylose line, GEMS-007, would translate into a protein with six amino acid polymorphisms relative to a lower amylose line, H99ae. Four of the amino acid polymorphisms are unique to the GEMS-0067 allele, but two are found in grass relatives of maize, but not in other maize varieties that have thus far been characterized. A review of the published *sbe1* sequence data indicates that most varieties of maize are remarkably uniform, i.e., they have the same amino acid usage as found in H99ae. We are gathering sequence data from more diverse maize varieties in order to survey the range of polymorphisms for this gene. We are curious if the uniformity found in so many maize varieties might have resulted from selection or fixation due to a genetic bottleneck. We present sequence data obtained through PCR amplification and sequencing for *sbe1* from several Native American varieties.

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P125

Sequence-enabled Genetics in Setaria viridis, a Model System for Panicoideae

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The subfamily Panicoideae contains many of the world's most productive cereal grasses, including maize and sorghum. However, they are large and long-lived, which impedes efforts to identify and validate gene candidates. Setaria viridis is a tractable model for rapid gene candidate analysis due to its short life cycle and small stature. We generated mutant populations for forward and reverse genetics. We conducted an NMU-mutagenesis, generated and characterized approximately 3000 mutant families, and are developing TILLING population. We identified several putative phenotypes similar to classical maize mutants in crucial biochemical pathways, including carbon allocation (zebra crossbands, narrow leaf, virescent,) and panicle development. A total of 55 mutant individuals with interesting phenotypes are being sequenced at ~30x coverage by JGI-DOE to empirically determine mutation frequency. Thus far, we observed that the average number of non-synonymous disruptive mutations was 50 per individual. In addition, a panicle mutant has been crossed with S. viridis accessions to fine map the causal gene using Bulk Segregant Analysis (BSA) followed by deep sequencing. To map drought tolerance related QTLs, we also initiated the construction of six recombinant inbred populations by crossing A10.1 to diverse S. viridis accessions, including a drought tolerant line, Roche 10106, from which a RIL population of 298 individuals is being developed through Single-Seed-Decent. Furthermore, we assembled a diverse germplasm collection of 430 S. viridis accessions in collaboration with other contributors. A subset of accessions is being sequenced at JGI-DOE to establish a panel for population genetic analyses and genome wide association studies (GWAS). We demonstrate that phenotypes of interest can be mapped to fine resolution with a thorough characterization of the standing phenotypic variation in a subset of this panel. To date, 60 lines have been characterized for phenotypic traits and have been propagated for seed distribution at the USDA GRIN (http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?430573).

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P126

Suberin feruloylation is not required for CO_2 concentration in the maize bundle sheath

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C_4 grasses often outperform C_3 species under hot, arid conditions due to superior water and nitrogen use efficiencies and lower rates of photorespiration. A method of concentrating CO_2 around the site of carbon fixation in the bundle sheath (BS) is required to realize these gains. In NADP-malic enzyme (NADP-ME)-type C_4 grasses such as maize, suberin deposition in the BS cell wall is hypothesized to facilitate CO_2 concentration by acting as a diffusion barrier to CO_2 escape and O_2 entry from surrounding mesophyll cells. Suberin is a heteropolyester comprised of acyl-lipid-derived aliphatic and phenylpropanoid-derived aromatic components. Suberin is synthesized by a large network of biosynthesis and regulatory genes, none of which have been characterized in any C_4 monocot to date.

We identified a set of candidate maize genes expressed concurrently with BS suberization and assembled a putative biosynthetic pathway based on functional characterizations from Arabidopsis. To disrupt aromatic suberin biosynthesis, we mutated two paralogously duplicated, unlinked maize homologues of Arabidopsis *ALIPHATIC SUBERIN FERULOYL TRANSFERASE*, *ZmAsfta* and *ZmAsftb*, using closely linked *Ds* transposons. We screened 7,200 testcross progeny of *Ds* donors 109kb and 44kb from *ZmAsfta* and 5,000 progeny of *Ds* donors 10kb from and within *ZmAsftb*. Four alleles were recovered at each locus and crossed to generate a series of double mutants. Characterization of loss-of-function double mutants revealed a 50-96% reduction in suberin-specific aliphatic monomers and an attenuated accumulation of osmiophilic material in the BS suberin lamellae. However, there were no other morphological phenotypes in photosynthetic tissues under ambient growing conditions. Furthermore, the mutation had no significant effect on net CO_2 assimilation at sub-ambient, ambient, or elevated CO_2 levels, and no effect on ^{13}C isotope discrimination relative to wild type. Thus, normal bundle sheath suberization is not required for NADP-ME C_4 photosynthesis in maize, contrary to existing models of CO_2 concentration.

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P127

Targeting the role of benzoxazinoid genes in maize-aphid resistance

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Benzoxazinoids are compounds toxic to aphids and other insects found in maize and other grasses stored as inactive glucosides. Benzoxazinoids are activated by a glucosidase upon herbivore feeding. Previous research identified a Quantitative Trait Locus (QTL) on Chromosome 1 in maize that is thought to affect benzoxazinoid biosynthesis. Three genes within the QTL (Bx10a-c) were identified as methyltransferases and are thought to catalyze the methylation of DIMBOA-Glc (a benzoxazinoid) to HDMBOA-Glc. To prove that these genes play a role in benzoxazinoid biosynthesis, we attempted to isolate loss-of-function knockout alleles induced by the transposable element Dissociation (Ds). We took a reverse genetic, PCR-based approach to screen for Ds insertions in the three target genes, as well as two intermediate genes. A screening population was generated by crossing a line known to carry a closely linked Ds insertion crossed to wild-type W22 inbred females. Should a Ds insertion be found within Bx10a-c we will self pollinate that plant and use the progeny in an aphid assay. Should an insertion be found surrounding Bx10a-c we will self pollinate that plant and use the progeny as new Ds donors in future test cross populations. Through reverse genetic screens of testcross populations, we hope to remobilize the closest Ds donor into the three gene targets (Bx10a-c) in order to compare the knockout alleles to a wild type in terms of benzoxazinoid synthesis.

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P128

The *brown midrib2* and *brown midrib4* mutants of maize link lignin biosynthesis to methylation and polyglutamylation

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Lignin is a heteropolymer component of plant cell walls that provides structural strength and contributes to resistance to some pests and pathogens. The maize *brown midrib (bm)* mutants, named for their reddish-brown midribs, accumulate less and altered lignin relative to non-mutants. Four of the six currently characterized *bm* mutants have been cloned and shown to encode enzymes in the lignin biosynthesis pathway. While *bm1* and *bm3* are involved in the direct synthesis of lignin, *bm2* and *bm4* reveal a previously uncharacterized link between lignin synthesis and components of the methylation and polyglutamylation pathways. The *bm2* gene encodes a functional methylene tetrahydrofolate reductase (MTHFR) involved in the formation of the methyl donor S-adenosyl-L-methionine, which is consumed by lignin pathway enzymes. The *bm4* gene encodes a functional folylpolyglutamate synthase (FPGS) that catalyzes the polyglutamlyation of folate compounds upstream of *bm2*-encoded MTHFR. The *bm2* gene was cloned via traditional mapping, RNA-sequencing, and BSR-Seq (Tang *et al.*, Plant J 2014). The *bm4* gene was cloned via a combination of traditional mapping, Seq-Walking, and PCR-based sequencing methods (Li *et al.*, in press). Mutants in the *bm2* and *bm4* genes accumulate 7-14% less lignin in stalks than their wild-type siblings at anthesis and senescence. For both mutants, the ratio of syringyl to guaiacyl lignin is elevated in mutant stalks. Functionality of the *bm2* and *bm4*-encoded enzymes was confirmed via yeast complementation studies. Paralogous genes of *bm2* and *bm4* have been identified that may explain the viability of these mutants. However, the functional overlap among paralogs is not complete, as evidenced by the alterations in lignin content in both mutants.

P129

The genetic interactions between brassinosteroid and gibberellic acid biosynthetic mutants are developmentally specific.

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Phytohormone regulation of plant architecture has been extensively studied. These studies typically consider a single hormone and a component of plant architecture, such as plant height or branching. As a result we still do not understand how phytohormones interact. Both brassinosteroids (BRs) and gibberellic acid (GA) affect plant height and sex determination in maize. We investigated the genetic interaction between *na2* and a GA biosynthetic mutant, *d5*. We identified the molecular basis of the *nana plant 2* (*na2*) phenotype as a loss-of-function mutation in the maize ortholog of the Arabidopsis BR biosynthetic gene DWF1. These mutants accumulate the DWF1 substrate 24-methylenecholesterol and exhibit decreased levels of downstream BR metabolites. Both *na2* and *d5* are severe dwarfs. *d5* tillers profusely and induces stamen production in the female ear but *na2* has pistil production in the male tassel. Double mutant analysis of *na2d5* indicated additivity for some phenotypes and epistasis for others with no unifying pattern. Disruptions to BR and GA biosynthesis influence height additively. Yet *na2d5* double mutants do not tiller, demonstrating that a functional *na2* was necessary for *d5* to induce tiller elongation. The development of male or female floral identities were controlled in different. In female flowers, *na2* did not suppress *d5* stamen induction, indicating that BRs were not required for GA to have this effect. In male flowers the *d5* mutation blocked the induction of pistil formation from the disruption in BR biosynthesis, demonstrating that GAs are required for loss of BR biosynthesis to affect a tassel seed phenotype. Similar results were observed between other BR and GA biosynthetic double mutants. These findings demonstrate that BR and GA do not interact in a single inclusive pathway and that differential signal transduction and downstream effects are dependent upon the developmental context.

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P130

The maize death acids, 10-oxo-11-phytoenoic acid and derivatives, demonstrate specificity in jasmonate-related signaling and defense

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Plant cellular damage promotes the interaction of lipoxygenases (LOX) with free fatty acids to yield 9- and 13-hydroperoxides which are further metabolized into diverse oxylipins. The enzymatic action of 13-LOX on linolenic acid enables production of 12-oxo-phytodienoic acid (12-OPDA) and its downstream products, jointly known as jasmonates. As signals, jasmonates have related yet distinct roles in the regulation of plant resistance against insect and pathogen attack. An additional and conceptually parallel pathway involving 9-LOX activity on linoleic acid leads to the production of 10-oxo-11-phytoenoic acid (10-OPEA). Despite structural similarity to jasmonates, physiological roles for 10-OPEA have remained unclear. In developing maize (Zea mays) leaves, fungal infection by Southern leaf blight (Cochliobolus heterostrophus) results in the localized production of 10-OPEA and a series of related 12- and 14-carbon metabolites, collectively termed ‘death acids’. While typically absent, 10-OPEA becomes highly wound-inducible within fungal-infected tissues. As a direct defense, 10-OPEA suppresses the growth of mycotoxigenic fungi, including Aspergillus flavus and Fusarium verticillioides, and also the insect herbivore Helicoverpa zea. Both 12-OPDA and 10-OPEA equally promote the transcription of numerous defense genes encoding glutathione S-transferases, cytochrome P450s, and pathogenesis-related proteins; however, 10-OPEA activity diverges in the context of reduced protease inhibitor transcript accumulation. Consistent with a role in dying tissue, 10-OPEA exhibits significant potency and specificity in triggering ion leakage and cell death, which is significantly impaired by the cysteine protease inhibitor maize cystatin-9. Unlike widely encountered jasmonates, functions of 10-OPEA and associated death acids are consistent with specialized roles in local defense reactions.

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P131

The Maize Genome Encodes Novel Members Of The Purple Acid Phosphatase Subgroup Ia

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Plant purple acid phosphatases (PAPs) play an important role in plant phosphorous nutrition, both by liberating phosphorous from organic sources in the soil and by modulating distribution within the plant throughout growth and development. Furthermore, members of the PAP protein family have been implicated in a broader role in plant mineral homeostasis, stress responses and development.

We have identified 33 candidate PAP encoding gene models in the maize (*Zea mays* ssp. *mays* var. B73) reference genome. The maize PAP family includes a clear single-copy ortholog of the *Arabidopsis* gene *AtPAP26*, shown previously to encode both major intracellular and secreted acid phosphatase activities. Certain groups of PAPs present in *Arabidopsis*, however, are absent in maize, while the maize family contains a number of expansions, including a distinct Subgroup, not present in *Arabidopsis*.
Inspection of RNA-sequencing based transcriptome data revealed accumulation of *Pap* transcripts in multiple plant tissues at multiple stages of development, and increased accumulation of specific *Pap* transcripts under low phosphorous availability. Our analysis reveals the maize PAP family to have broad general importance throughout the plant life cycle, while highlighting potential functional specialization among individual family members.

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P132

The role of ZmAMP: an Antimicrobial Protein from Maize

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ZmAMP is a maize gene that encoding a putative 115 amino-acid antimicrobial peptide, its sequence and structure model remains highlighted similarity to MiAMP1 and other proteins that inhibits the growth of several microbial plant pathogens *in vitro*, these proteins forms a new class unique amongst plants antimicrobials called β-barrelins. Interestingly and paradoxically *ZmAMP* expression apparently is induced in corn root colonized by arbuscular mycorrhizal fungi, its mutualistic symbiont. *ZmAMP* is in maize line B73, while line Mo17 has a partial absence, so there is a Presence-Absence Variation and means advantages for experimental designs using plants with *ZmAMP* locus introgressions. In this research we focus to determine the effect of recombinant protein on microorganisms *in vitro* and gradually to assign a role of ZmAMP in mycorrhizal plants. It could be considered a potentially useful tool for genetic improvement to obtaining maize varieties with pathogen resistant feature.

Funding acknowledgement: CONACYT

P133

The Success Strategies of a Bursting Transposon

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Transposable elements (TEs) comprise the largest proportion of all characterized plant and animal genomes. In part, this reflects the ability of a few TEs in a genome to undergo a "burst" – a term that describes the rapid increase in number to thousands, even tens of thousands of copies. In plants, two TE types are associated with bursts: class 1 LTR retrotransposons and class 2 miniature inverted repeat transposable elements (MITEs). While the former have a tendency to insert in intergenic regions, usually into other LTR retrotransposons, MITEs attain copy numbers of hundreds and thousands despite a preference for genic regions. How MITEs do this without killing their host or being silenced is the focus of our studies.

Although MITEs were first discovered in maize, rice (*Oryza sativa*) has proven to be our organism of choice for determining their strategies for success. First, the small genome of rice facilitated the computer-assisted discovery of *mPing*, the first actively transposing MITE and Ping, the autonomous element responsible for its movement. A strategy for *mPing*'s ability to rapidly increase in copy number was revealed to be a preference for insertion into noncoding genic regions and an avoidance of exons. Second, as a predominantly self-pollinating organism, pure lines of rice can be propagated for decades. We have exploited this feature to determine the genome-wide impact of *mPing* bursts in two pairs of rice strains, each derived recently from a common ancestor: EG4/HEG4 and A123/A119. Comparative analysis of their sequences provide evidence that (1) the strain pairs have been maintained as pure lines for ~20 and ~100 years, respectively, (2) the bursts have been sustained for decades in the presence of what appears to be normal genome surveillance, and (3) the bursts were probably not initiated by what McClintock called "genome shock".

P134

Transcriptional Regulatory Network Controlling Phenolic Biosynthesis in Maize

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Maize accumulates large numbers of phenolic compounds, such as lignins and flavonoids, which play important roles in plant growth and adaptation. Lignins are crucial for biomass production and flavonoids are key nutraceuticals providing value to human and animal diets. The goal of this study is to identify the gene regulatory network (GRN) that controls phenolic biosynthesis in maize. First, to accurately define the transcriptional regulatory regions, we performed Cap Analysis of Gene Expression (CAGE) to identify genome-wide transcription start sites (TSSs) used in root and shoot tissues of two widely utilized maize inbred lines (B73 and Mo17). Based on CAGE data we cloned promoters of 56 phenolic genes as 1kb in length upstream of TSSs. These promoters were used as baits in yeast one-hybrid assays (Y1H) to screen a library containing 1901 maize transcription factors (TFs). Totally, 1614 protein-DNA interactions (PDIs) were newly identified between 732 TFs and 56 promoters.
Six TFs bind 9 to 16 promoters, which might be master regulators. Next, to evaluate the possibility of PDIs *in vivo*, co-expression analyses were performed between enzymes and TF-enzymes, using microarray and RNA-Seq data across a large range of maize tissues and developmental stages. We found that some enzymes could be acting as complexes and are likely co-regulated by a group of TFs. Finally, around 50 TFs, which co-expressed with their binding enzymes, were selected for further study *in vivo* by Chromatin immuno-precipitation (ChIP) and transient expression assay. These data will help to elucidate the cis-regulatory elements and regulators that control phenolic biosynthesis in maize, providing unique functional insights into the regulatory mechanisms of phenolic biosynthesis.

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P135

Transcriptomic complexity of maize primary root tissues in response to low water potentials

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Water deficit is one of the most severe abiotic stresses limiting maize growth and productivity worldwide. A detailed understanding of the molecular mechanisms underlying the water deficit response of maize will enable targeted breeding strategies to develop drought tolerant cultivars.

The early transcriptional response of maize (*Zea mays* L.) primary root tissues to low water potentials was analyzed in this study.

Young maize (B73) seedlings were subjected to low water potential (-0.8 MPa) or control treatment for 6 hours. The transcriptomes of the four root tissues meristematic zone (1), elongation zone (2), and cortex (3) and stele (4) of the differentiation zone were then analyzed separately by RNA-sequencing. In pairwise comparisons of low water potential and control treatment, water deficit responsive genes for each root tissue were computed. Most genes were differentially expressed in the cortex of the mature root zone (5,789) and in the elongation zone (4,403), while fewer genes were affected in meristematic zone (3,095) and stele (1,823). Most differentially expressed genes across all tissues were up-regulated upon water deficit. For genes that were responsive in more than one tissue the direction of regulation was conserved. The high degree of plasticity of the water deficit response in primary roots was illustrated by the observation that 71% of all responsive genes were tissue-specifically regulated. Functional categorization revealed overrepresentation of processes of transcriptional regulation in all tissues and of further tissue-specific categories, such as cell wall metabolism in apical tissues.

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P136

Transgenic control of aflatoxin contamination in corn through host induced gene silencing

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Maize (*Zea mays* L.) is one of the major crops susceptible to *Aspergillus flavus* infection and subsequent contamination with aflatoxins, the most potent naturally produced carcinogenic secondary metabolites. This pathogen has the potential to cause severe economic losses due to aflatoxin contamination. The *A. flavus* polygalacturonase 2c (P2c), a key enzyme involved in the colonization of maize kernels, was selected as a possible candidate for suppression through host induced gene silencing (HIGS). In this study, an RNAi vector containing the P2c gene was constructed and introduced into the immature B104 maize embryos through *Agrobacterium* transformation. Fifteen transgenic plants from fifteen independent transformation events were produced. PCR analysis of the genomic DNA from leaf tissue confirmed the presence of transgene in twelve out of the fifteen plants. Real time PCR analysis of RNA isolated from transgenic leaf tissues also showed a high variation in fungal target gene expression among the transgenic leaf tissues. Kernel screen assays were performed to determine aflatoxin level in the mature transgenic seeds seven days after inoculation with *A. flavus*. It was found that maize kernels containing the P2c gene had less aflatoxin than those that did not contain the gene. The results from these preliminary studies suggest that the HIGS using P2c gene can reduce aflatoxin contamination in maize. Future studies will focus on detecting the presence of gene specific siRNA.

Funding acknowledgement: United States Department of Agriculture (USDA), The research was also supported by Aflatoxin Mitigation Center of Excellence (AMCOE)

P137

Transgenic expression of the maize benzoxazinoid biosynthesis in *Arabidopsis thaliana*

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Plants collectively synthesize a huge variety of secondary metabolites that constitute the arsenal of chemical defense. The control of diseases and reduction of damage by herbivory is essential for plant performance and yield. Often specific defense compounds are restricted to members of one plant family. Benzoxazinoids are constitutive defense metabolites synthesized in the grasses, the major benzoxazinoid in maize is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA). Protection of the young maize plant against microbial pathogens and herbivory (e.g. the European corn borer) is well documented. All maize biosynthetic genes (*Bx*-genes) have been isolated. Secondary biosynthetic pathways branch off primary metabolism via signature enzymes. This link allows transgenic expression of specific pathways in any given plant thereby potentially increasing the plant's defense repertoire. Transgenic expression of the defense compound on the other hand might directly (toxicity) or indirectly (metabolic bottlenecks) impact the plant's fitness. We have generated transgenic Arabidopsis expressing the first genes of DIMBOA biosynthesis. A comprehensive metabolomics and transcriptomics analysis has been performed and biotic interactions were analyzed.

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P138

Understanding the Molecular Mechanisms of Maize Response to Abiotic Stress Factors

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As global warming becomes more prominent, there is increased threat to the sustainability of corn production due to the crop's inability to withstand changes in abiotic factors of drought, salinity, ultraviolet radiation damage, and temperature fluctuation(Mittler, 2006). Of late, there have been findings that maize may have the ability to respond to stress factors faster if previously exposed to the factor. In a recent study of recurring dehydration stresses, Arabidopsis demonstrated "trainable genes", where increased rates of transcription of stress-response genes were triggered(Ding, 2012). Additionally, Sung and colleagues(2003) found improvements in chilling tolerance through modification of transcription factors. These sources indicate that previous exposure can result in decreased susceptibility to damage from abiotic stress.

This experiment included subjecting maize lines B73, Mo17, Oh43, and MoG to various abiotic stress factors to document general susceptibility. Another aim was to discern whether early exposure to cold-stress would result in decreased susceptibility to damage in maize plants, and to identify possible maize effector genes assisting in the decrease of damage to "primed" plants. In order to do this, a "priming" protocol was developed, wherein Mo17 and MoG plants were exposed to cold at both days 8 and 9 of development. These "primed" plants and plants that were not "primed" were subjected to cold-stress on day 14 of development. After cold-stress, plant appearance was observed and leaf tissue collected. RNA was extracted from leaf tissue and converted to cDNA. Finally, qPCR was performed to analyze the transcription rates of (GRMZM2G124011, GRMZM2G415973, AC210204.3_FG002, and GRMZM2G009683) genes in response to cold-stress.

Although significant differences in appearance were not visible, variation of transcription rates of selected genes between "primed" and control plants suggest success of "priming" concept. Further investigation regarding "priming" and cold-stress will be conducted, including RNA-sequencing of "primed" and cold-stressed plants.

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P139

Understanding the protective role of the maize silk surface lipid metabolome against water stress

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The aerial portions of a plant are protected by a cuticle that is embedded with and coated in a complex array of non-polar lipids (i.e. the surface lipid metabolome) and is thought to function as a protective water barrier between the plant and its environment. A hydrophobic cuticle protects the stigmatic maize silks, which are composed of ~90% water and are frequently exposed to water stress during the critical period of pollination. Using a metabolomic approach we have previously demonstrated that silk surface lipids consist primarily of very-long chain saturated and unsaturated hydrocarbons, fatty acids and aldehydes ranging from 16 to 35 carbons in length. We observe that relative abundances of these hydrophobic metabolites differ among maize genotypes (e.g. B73 vs. Mo17) and along the lengths of silks. For example, silks that are exposed to the external environment (i.e. emerged silks) can accumulate 3- to 5-fold more surface lipids than the portions of silks that are covered by husk leaves (i.e. encased silks). To better understand the protective capacity of the silk surface lipid metabolome against water stress, we are assessing rates of silk water loss from excised silks exposed to different humidity treatments (relative humidity = 15% and 85%) at a constant temperature, which generates different vapor pressure deficits (VPD). We will present initial studies that optimize the experimental system and examine rates of water loss in emerged and encased silks exposed to both high and low humidity treatments. These studies will allow assessment of specific surface lipid metabolomes as water barriers during water stress.

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P140

Understanding the Role of Dihydroflavonol 4-reductase Substrate Specificity and Promiscuity in Flower Color Regulation

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Plants, unlike other organisms, cannot move and thus have evolved unique ways of adapting to their environment. These adaptations include the production of specialized compounds called secondary metabolites. Flavonoids, a class of secondary metabolites, are abundantly represented in angiosperms, displaying a diverse spectrum of biological functions in plants, which include coloration and UV protection; they may also provide health benefits to humans, such as reduced risk of heart disease and cancer. A subgroup of flavonoids, anthocyanins, are responsible for the primary pigmentation of flowers. Dihydroflavonol 4-reductase (DFR), an enzyme in the anthocyanin biosynthetic pathway, helps create a range of flower colors due to its ability to catalyze the reduction of dihydroflavonol substrates into the precursors of their respective anthocyanins. This enzyme has evolved to favor specific substrates, leading to different flower colors. Biochemical analysis provides an additional perspective on the evolution of these secondary metabolites that phenotypic analysis cannot. Therefore, several orthologous DFR enzymes from *Amborella trichopoda, Cymbidium hybrida, Petunia hybrida, Iochroma baumii, Iochroma gesnerioides,* and *Arabidopsis thaliana* were recombinantly expressed. Out of the six, four were successfully purified, and enzymatic assays and crystallography were performed to further analyze their activities and substrate specificities. DFRs purified from *Iochroma baumii, Petunia hybrida*, and *Amborella trichopoda* all displayed preference for dihydromyricetin over dihydroquercetin. Initial DFR crystal screens were unsuccessful, therefore ThermoFluor assays were used to measure the stability of DFR in the presence of various substrates and a cofactor. NADPH does stabilize DFR, suggesting a cofactor is necessary for the enzyme to crystallize. Conclusively, secondary metabolites are a unique class of compounds that provide an evolutionary advantage for flowering plants. By understanding these metabolites using a biochemical approach, we can unravel another perspective for their evolution.

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P141

Wilty2, a β-tubulin6 protein, is required for biosynthesis of endodermis suberin and important for water absorption in maize

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The Wilted2 (Wi2) mutant is a semi-dominant mutant induced by EMS. Compared to wild type, the mutant is more sensitive to abiotic stresses, such as high temperature, drought stress and osmotic stress. The expression of ABA and H2O2 synthesis related genes were up-regulated under normal condition in Wi2 mutant. ABA and H2O2 concentration were increased in the leaves of Wi2 mutants, which resulted in the stomata closing. Genome-wide expression analysis using RNA-Seq showed that most gene expression level in Wi2 mutant under normal condition was equal to that of wild type under drought condition.
Map-based cloning result shows that Wi2 encodes β-tubulin6 with a point mutation within a conserved domain. The TUB6 of Wi2 mutant had one amino acid substitution: proline (P287) to leucine (L), which introduced one extra alpha-helix in mutant as compared to wild type, The alpha helix can likely affect the assembly of microtubules.
Histochemical analysis suggested that tub6 were mainly expressed in xylem, pith, phloem, pericycle and endodermis cells of the root maturation zone, but little or no expression was detected in root cap, division zone, elongation zone, stem, leaf and the cortex and epidermis of root maturation zone. UV-Fluorescence microscope observation of cross section of mutant and wild type crown root showed that the wall of endodermis was not thickened in Wi2 mutant. Total content of the aliphatic components of suberin in roots show a clear reduction in Wi2 which correspond to observation of a reduced UV-fluorescence. This change affected the transportation of water and nutrient from root to shoot. The mutation of TUB6 may affect the dynamic of microtubules which affect the suberin deposition. The later then affect the thickening of the endodermis walls.

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P142

Workflow and methods for high throughput genotyping of transgenic *Setaria viridis*, an emerging C4 model plant

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Setaria viridis is an emerging C4 model plant with the technical advantages of a diploid genome (~510Mb), small stature and rapid life cycle. As *S. viridis* transformation methods become more efficient, high throughput genotyping protocols are needed. In this study, methods for determining insertion copy number and transgene zygosity were developed for *S. viridis*, and multiple genotyping workflows were optimized. A Southern DIG hybridization protocol was optimized to determine insertion copy number, and a real-time qPCR assay was developed to determine transgene zygosity and verify copy number. Using these genotyping methods, multiple workflows were optimized. These methods and protocols could easily be extended to monitor transgene copy number in maize.

All workflows resulted in genotyped, transgenic *S. viridis* lines that were ready for downstream applications at the T_2 generation. The rates of single copy insertion events using different transformation vector backbones and different *S. viridis* germplasm were compared. A high through-put genotyping procedure, combined with a highly efficient transformation protocol will establish *S. viridis* as a leading model for molecular and biochemical studies in C4 grasses.

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P143

Zea mays Sucrose transporter2 contributes to plant growth, development, and agronomic yield

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During daylight, plants often have excess photosynthetic productivity, resulting in the accumulation of photosynthates, including sucrose that is transiently stored in the vacuole. At night, or as photosynthesis becomes limiting because of environmental conditions, plants can remobilize sucrose from the vacuole into the cytoplasm to sustain the plant's metabolism and growth. Based on homology to other SUT2 transporter proteins, *Sucrose transporter2* (*ZmSut2*) is hypothesized to function as a sucrose/H+ symporter located on the tonoplast membrane and to export sucrose temporarily stored in the vacuole. To understand the biological importance of *ZmSut2*, we identified several *Mutator* transposon insertions into the gene and characterized the resulting mutants using molecular and phenotypic analyses. From preliminary studies in the greenhouse in which wild-type and mutant plants were grown in the same pot, we observed that the mutants had a slower growth rate and were generally of a smaller stature. Based on these growth differences, wild-type and mutant plants were grown under increasing population densities in the field to investigate whether increased competition (i.e., reduced light interception) would exacerbate phenotypic differences. The plant growth rate tended to be slower in the mutants compared to their wild-type siblings throughout plant development, and this difference became more exaggerated at higher densities. Ear and kernel size were also significantly smaller in the mutants at higher plant densities. These findings suggest that *ZmSut2* plays an important role by remobilizing sucrose out of the vacuole for subsequent use in growing tissues, and that *ZmSut2* function makes an important contribution to maize development and agronomic yield.

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P144

ZmDCT2 has a major role in the photosynthesis development of maize

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C_4 photosynthesis is a complex metabolic pathway that utilizes specialized anatomical and biochemical mechanisms to concentrate CO_2 around RuBisCO, effectively eliminating photosynthetic losses associated with photorespiration. The circular metabolic activities of the C_4 pathway are coordinated between two specialized leaf cell types, mesophyll (M) and bundle sheath (BS), and rely heavily on active transport of metabolites. Although the action of transporter proteins is critical for the proper function of C_4 photosynthesis, the identity of most of these transporters remains elusive. *ZmDCT2* is a dicarboxylate transporter that can transport malate at high efficiency. It is differentially expressed in the BS cells in maize, has a photosynthetic gene expression pattern along the leaf gradient, and is light responsive, making it a good candidate for malate transport into the BS chloroplast during C_4 photosynthesis in maize.
We characterized the role of *ZmDCT2* in maize leaves via insertional mutagenesis. Using an *Ac* element, Ac.bti99221, that was located 129 kb (0.44 cM) from the gene. We screened 1050 high-dose kernels to identify 4 alleles - these included both strong and weak alleles. We show that *ZmDCT2* is expressed in the BS and is necessary for transport of malate into the BS chloroplast. We also show, via isotopic labeling experiments, that both WT and mutant maize leaves have an active PEPCK C_4 photosynthesis pathway that accounts for about 25% of the photosynthetic activity of the plant. Our results emphasize the importance of transport during C_4 photosynthesis and unravel the role of a key player in this complex pathway.

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P145

A fun new mutant affecting sex determination and leaf architecture

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The feminized, upright and narrow (fun) mutant is recessive, generated by EMS mutagenesis. The fun mutant tassel is incompletely feminized while all the adult leaves are 20% narrower than wild-type siblings. Leaves lack auricles, leading to the uprightness of the blade. Interestingly, the ligule is unaffected which distinguishes fun1 from all other maize leaf mutants that affect auricle development. The fun gene was cloned by chromosome walking, and the mutation in fun-ref results from an N terminal stop codon leading to a truncated protein. The FUN protein is of unknown function but is conserved in grasses, perhaps implying a conserved role in auricle and blade expansion in the grasses. On the other hand, FUN's role in sex determination in hermaphroditic grasses remains unclear. In order to answer the question of FUN's role in other non-monoecious grasses, mutants in Brachypodium distachyon and Sorghum bicolor are being isolated. Although the protein is of unknown function, a bipartite NLS sequence has been identified, as well as a neighboring serine rich region, which could imply phosphorylation control of nuclear localization. Nuclear localization will be confirmed by creation of an antibody, which will also allow determination of the developmental stage at which FUN is expressed. A silkless/fun double mutant displayed reduced tassel branching suggesting an additional role for FUN in tassel branch formation. The wide range of fun1-ref phenotypes seen in tassels and leaves indicate that conserved genetic pathways exist that control cell differentiation in both structures.

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P146

A new *barren* mutant in maize

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Maize produces two types of reproductive structures - the tassel and the ear. The tassel consists of a main spike with several long branches and is the male reproductive part of the plant. The ear shoot develops from the main stalk and is the female reproductive part of the plant. Both the tassel and ear are crucial in ensuring crop yield and hence studying their development is important in agriculture.

Several classes of mutant affect tassel and ear development. The *barren inflorescence* class of mutants is defective in tassel and ear development often due to defects in the plant growth hormone auxin. The *barren stalk (ba)* class of mutants is characterized by having no ear shoot and in some cases the tassel is also affected. The *ba1* and *ba2* mutants are in this class and both lack branches in the tassel and have no ears. Both *ba1* and *ba2* are proposed to act downstream of auxin in the development of tassels and ears.

A new *barren* mutant, *ba*CL* is presented that has been found to map to chromosome 6, between bin 6.04 and 6.05, and hence represents a new locus. The mutants have no ear and have fewer branches in the tassel. SEM analysis shows that the immature tassels of this mutant have defects that differ from either *ba* or *bif* mutants. Further characterization of the mutant is being performed to understand its role in development and fine mapping is being carried out in order to clone the gene.

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P147

An expression atlas of the maize shoot apex

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In plants, stem cell niches serve as a stable source of cells for postembryonic growth and development. The shoot apical meristem (SAM) gives rise to all aerial organs of a plant, and its activity throughout the plant's lifetime therefore has to be tightly controlled in a spatiotemporal manner. The slowly dividing stem cell population at the tip of the SAM is maintained throughout development and generates the founder cells for the repeated initiation of new organs. To gain insight into gene regulatory networks behind stem cell maintenance and organogenesis, we generated a high-resolution gene expression atlas of 10 distinct cell types within the vegetative maize shoot apex using laser microdissection and RNA deep sequencing. Using this data set, we identified cell type specific expression features and describe the transcriptional changes associated with the progression through organogenesis. Furthermore, we identified unique transcription factor signatures that, next to the interplay of hormones, distinguish stem cells from their differentiating descendants within the SAM. Our findings present a novel approach to modulating plant architecture by manipulating master regulators of cell identity.

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P148

Analysis of maize embryo morphogenesis in thirteen *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. Kernels from segregating ears were planted and crossed by B-A translocations to determine the location of the mutated gene. Several have been located to Chromosome arm 1S. 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 pro-embryo stage to Stage 2. The 13 mutants presented here represent as many as 13 loci that appear to specifically affect embryogenesis. For further analysis each of the *emb* mutants was crossed with fusion protein constructs to determine developmental patterns of proteins that may be involved with embryogenesis.

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P149

Analysis of the receptor like kinase WARTY2 and its role in epidermal patterning of bulliform-like cells in the maize leaf

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Maize leaves develop from the shoot apical meristem in a gradient of cell division, expansion and differentiation. The leaf epidermis is arrayed in a predictable linear pattern that is regulated by precise spatiaotemporal expression of developmental signals and their receptors. We previously identified a cell pattern mutant *warty2* (*wty2*) with overly expanded bulliform-like cells in the adaxial and abaxial epidermis of juvenile and adult leaves. Mutant *wty2* leaves are corrugated with excessive leaf rolling related to the excessive production of bulliform-like cells. The WTY2 gene encodes a novel receptor-like kinase (RLK) belonging to the LRR VII subfamily and is an inactive kinase. 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, and the FP signal showed polarized distribution in the plasma membrane. To characterize the WTY2 signaling pathways, *wty2* mutants were crossed to other cell pattern mutants. Possible synergistic effects were observed only between *wty2* and *cr4*, a mutant in another receptor kinase. To identify suppressors or enhancers of Wty2, EMS mutagenesis was conducted on *wty2* mutants. The M1 generation has been initially screened for dominant mutants and to date, three dominant chimeras have been identified that show a normal to mild mutant phenotype in a sector in an otherwise homozygous severe mutant plant. Sequencing of the phenotypically mild sector in one of the chimeras uncovered a mutation that introduces a stop codon in one allele of the mutant gene. These results suggest that dosage of the *wty2* mutant allele affects the patterning phenotype. The UniformMu population was screened for insertions in genes in the same LRR subfamily of Wty2. Five genes were identified with insertions, but none showed a visible *wty2* phenotype. Co-immunoprecipation studies using WTY2-YFP are currently underway: mass spectrometry will be performed, and potential partners identified. The current data implicate WTY2 as a new LRR-RLK involved in transduction of a positional signal that controls maize leaf epidermis differentiation.

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P150

Analysis of the relative protein expression of TB1 and GT1 in maize versus teosinte

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Ideal plant architecture is often obtained by alteration of axillary bud activity. For example, suppression of tillering is one of the most frequently targeted morphological changes selected in modern crops during domestication from their wild ancestors. In maize, two of the best characterized tillering regulators are *teosinte branched1* (*tb1*) and *grassy tillers 1* (*gt1*). These two distinct transcription factors function in the same pathway, with *gt1* acting downstream of *tb1*. To further understand how the *tb1*/*gt1* pathway has been modified in maize versus teosinte, we recently raised TB1 and GT1 antibodies to characterize their expression in the two plants. Full-length maize TB1 and GT1 proteins were used as antigens, followed by purification using their respective unique C-terminal peptides. The specificity of the antibodies was confirmed through western blots and immunolocalization. Both TB1 and GT1 protein localize to axillary buds, especially in the surrounding immature leaf primordia, but much less in the axillary meristem. Interestingly, TB1 and GT1 show lower expression levels in teosinte axillary buds compared to maize, consistent with both genes being selected together as domestication targets that function in the same pathway.

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P151

Autophagic Recycling Plays a Central Role in Maize Nitrogen Remobilization

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Macroautophagy (hereafter autophagy) is a primary route for nutrient recycling in plants by which superfluous/damaged cytoplasmic material, large protein complexes, and organelles are encapsulated into double membrane-bound vesicles and delivered to the vacuole for breakdown. Central to autophagy is a conjugation system that attaches phosphatidylethanolamine to AUTOPHAGY-RELATED (ATG)-8, which then coats emerging autophagic membranes and helps promote cargo recruitment, vesicle enclose, and subsequent vesicle docking with the tonoplast. A key component in ATG8 lipidation is ATG12 that provides the ligase activity upon its covalent attachment to ATG5 and subsequent assembly into a ATG12-ATG5/ATG16 complex. Here, we more fully defined the maize ATG system by transcriptome analysis and characterized it genetically through *ZmAtg12* mutants that block ATG8 lipidation. RNA-seq analysis revealed that the mRNA levels of most core ATG components are significantly increased in senescing leaves/tissues and in developing endosperm, suggesting a specific role in these organs. Two *UniformMu* insertion mutants were identified in *ZmAtg12* that delete the C-terminal end of the resulting protein and thus block its attachment to ATG5. Plants missing *ZmAtg12* have compromised autophagic transport as determined localization of the YFP-ATG8a reporter and its vacuolar cleavage during nitrogen or fixed-carbon starvation. Phenotypic analyses showed that the *atg12* plants are phenotypically normal and fertile when grow under nitrogen-rich conditions. However, when nitrogen starved, seedlings growth is severely retarded, and as the plants mature, they show enhanced leaf senescence and delay ear development. Nitrogen partitioning studies revealed that remobilization is significantly impaired in *atg12* plants, which decreases seed nitrogen content and yield. Together, our studies demonstrated that ATG8-mediated autophagy is not essential to maize, but becomes critical to growth and development during nitrogen starvation by helping promote nutrient recycling, and thus should severely impacts crop productivity under suboptimal field conditions.

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P152

Auxin signaling in the Antipodal Cells of Maize Embryo sac

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The angiosperm female gametophyte, or the embryo sac, has four cell types: the two synergids, the egg cell, the central cell, and the antipodal cells. The synergids attract the pollen tube; the egg and central cell are fertilized by sperm nuclei to produce the embryo and endosperm, respectively; but the antipodal cell function remains unclear. In maize and many other grasses, the antipodal cells proliferate to produce a distinct cluster at the chalazal end of the embryo sac prior to fertilization. Accumulation of auxin in the antipodal cells was confirmed using *PIN1::PIN1-YFP* and the auxin reporter *DR5::RFP*. Analysis of Embryo sac transcriptomes confirms the presence of different gene families involved in auxin biosynthesis, distribution and signaling. In contrast to presence of auxin in the antipodal cells, expression of a cytokinin reporter was observed adjacent to but outside of the antipodal cells. In *Laxmidrib1*, a dominant leaf polarity mutant with reduced antipodal cell proliferation, expression of auxin reporters *PIN1* and *DR5* was absent. Another mutant with reduced antipodal cell proliferation also lacks *PIN1* expression in the antipodal cells. In mutants a correlation was observed between loss of auxin signaling with loss of antipodal cell proliferation. Additional experiments to determine the function of antipodal cells and its role in seed size are in progress.

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P153

Barley Six-Rowed Spike 4 (Vrs4), the ortholog of maize RAMOSA2, controls spikelet determinacy and row-type

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Inflorescence architecture of barley (Hordeum vulgare L.) is common among the Triticeae species, which bear one to three single- or multi-flowered spikelets at each rachis node. Triple spikelet meristem (TSM) is one of the unique features of barley spikes, in which three spikelets (one central and two lateral spikelets) are produced at each rachis node. Fertility of the lateral spikelets at TSM provides row-type identity. Six-rowed spikes show fertile lateral spikelets and produce increased grain yield per spike, compared to two-rowed spikes with sterile lateral spikelets. Thus far, two loci governing the row-type phenotype were isolated in barley that include Six-rowed spike1 (Vrs1) and Intermedium-C (Int-C). In the present study, we isolated Six-rowed spike4 (Vrs4), a barley ortholog of the maize (Zea mays L.) inflorescence architecture gene RAMOSA2 (RA2). Eighteen coding mutations in barley RA2 (HvRA2) were specifically associated with lateral spikelet fertility and loss of spikelet determinacy. Expression analyses through mRNA in situ hybridization and microarray showed that Vrs4 (HvRA2) controls the row-type pathway through Vrs1 (HvHox1), a negative regulator of lateral spikelet fertility in barley. Moreover, Vrs4 may also regulate transcripts of barley SISTER OF RAMOSA3 (HvSRA), a putative trehalose-6-phosphate phosphatase (T6PP), involved in T6P homeostasis implicated to control spikelet determinacy. Our expression data illustrated that although RA2 is conserved among different grass species, its down-stream target genes appear to be modified in barley and possibly other species of tribe Triticeae.

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P154

Beyond the wall: characterizing the role of boron in the meristem

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The element boron (B) is an essential plant micronutrient, and B deficiency results in significant defects in vegetative and reproductive development in plants. The maize *tassel-less1* (*tls1*) gene encodes a B transporter in the aquaporin family, and *tls1* mutants have altered inflorescence development, as well as defects in vegetative growth, including leaves and roots. Analysis of B content in *tls1* mutants reveals an overall reduction in B concentration, with the greatest deficit observed in immature inflorescences, indicating that meristematic tissues are particularly sensitive to B deficiency. SEM and histological analysis of *tls1* vegetative and inflorescence apices show that *tls1* mutants have smaller shoot and inflorescence meristems, suggesting that B plays a role in meristem maintenance. B is best known for its role in cross-linking the cell wall polysaccharide rhamnogalacturonan-II (RG-II). The level of dimerized RG-II is significantly reduced in young *tls1* tassels, indicating that the meristem defects may result from altered cell wall properties. However, there is no difference in RG-II cross-linking in *tls1* leaves or roots, suggesting that B plays additional roles in the plant. Historically, there have been reports of B interacting with hormones, namely auxin and cytokinin; however, the specific nature of these relationships remains unresolved. *tls1* mutants have root defects similar to those seen in cytokinin signaling mutants, and recent studies in other plants show that low B affects the transcription of cytokinin signaling genes. Additionally, our analysis of double mutants between *tls1* and cytokinin or auxin mutants further supports an interaction between B and hormones. We propose that cross-talk between auxin, cytokinin, and B plays a critical role in plant development, and current work is focused on using molecular markers and genetic tools to dissect the relationship between hormones and boron in the meristem.

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P155

Characterization of miR319-Regulated TCPs in Maize Inflorescence Development

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Maize produces two inflorescences, the tassel and the ear, that are essential for reproduction. Both inflorescences arise from similar inflorescence primordia and are patterned largely by the same developmental regulators. Some of these inflorescence regulators are also responsible for leaf development and are critical for establishing plant architectures. TEOSINTEBRANCED1/CYCLODIA/PCF (TCPs) are a unique class of plant-specific transcriptions factors that control proliferation and differentiation to establish plant architecture. A subset of TCPs (CIN-TCP) are regulated by microRNA (miRNA) miR319 and are required for petal and leaf development in multiple plant species. miRNAs are short non-coding RNAs that direct cleavage of target mRNAs. The maize *fuzzy tassel* (*fzt*) mutant is a hypomorphic allele of *dicer-like1*, which encodes a key enzyme required for miRNA biogenesis. *fzt* mutants have a broad range of vegetative and reproductive defects including reduced plant and leaf size, meristem indeterminacy in the inflorescences, and sex determination defects. *fzt* has reduced levels of some miRNAs, including miR319. Because miR319 is significantly reduced in *fzt* mutants and *TCP* genes have well-known roles in plant development, we hypothesized that misexpression of *TCP* genes might contribute to the *fzt* phenotype. RNA-seq analysis of tassel primordia indicated that CIN-like *TCPs* were expressed at similar levels in *fzt* mutants and normal siblings. RNA-seq at the whole tissue level cannot detect changes in expression domain or timing, however, so we are also examining the spatiotemporal expression of miR319-targeted *TCPs* in shoot apices and tassel primordia using RNA in situ hybridization. Preliminary data suggests that at least two *TCP* genes are expressed in all inflorescence meristems and floral primordia. We are currently confirming these results and also examining expression in *fzt* mutant inflorescences.

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P156

Characterization of the maize boron efflux transporter family

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Boron is an essential micronutrient for plant growth and development and plays an important role in the structure and maintenance of plant cell walls. Proper boron nutrition is critical for obtaining high yields in crop species. We recently reported the characterization of the boron efflux transporter RTE (ROTTEN EAR). Strong alleles of *rte* mutant produce tassels devoid of spikelets and ears that appear to decay during development.

To further understand how boron is transported and distributed during vegetative and reproductive development, we have identified six additional boron transporter genes in the maize genome (*rte2*-*rte7*). The transcripts abundance of the *rte* family members differs in terms of tissue specificity. The *rte* mRNA is present in all the tissues examined including leaf, root, ear, tassel and pollen, it is most abundant in ears. Interestingly *rte2*, despite having 94% identities at nucleotide level with *rte*, maintains low steady-state transcript levels in all the tissue tested, while *rte5*, *rte6* and *rte7* expression are only restricted to pollen.

We observed that both RTE and RTE2 can functionally complement the Arabidopsis *bor1* mutant. However, contrary to *rte*, mutations in the *rte2* gene do not show any growth defects. We constructed double *rte;rte2* mutants and we observed a strong enhancement of the *rte* phenotype when grown in boron deficient conditions. The *rte;rte2* double mutant plants showed stunted growth with only 6-7 leaves produced, and collapsed shoot apical meristem. These plants never produced any reproductive structures. Further work is in progress to dissect the role of individual boron transporters, as well as their coordinate action in the uptake and distribution of boron during maize development.

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P157

Characterization of the Maize *tangled-1* Mutant and TAN Interacting Partners

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Plant cell division is extremely diverse in many ways. During the onset of early division, a structure named the pre-prophase band forms and is thought to be a marker for the division plane which will be formed. Later in the division, a complex of microfilaments, microtubules, and endoplasmic reticulum elements forms the phragmoplast, which will lay down the foundation for the cell plate which ultimately guides the formation of the cell wall. *tangled-1* mutants in maize exhibit misoriented division planes. Though many of the cells in the tangled-1 mutants have atypical shapes and strange orientations, the overall shape of the maize plant is maintained. TAN has been show to localize to the cortical division site at least twice during division and is sustained at this location throughout division. Since tan mutants exhibit misoriented division planes and TAN is localized at the cortical division site, TAN can be used as a candidate for identifying new proteins required for plant cell division as well as division orientation. We are addressing the role that TAN is playing through careful analysis of maize *tan* mutants using time-lapse imaging as well as mathematical modeling. We will also be taking more biochemical approaches into characterizing proteins that interact with TAN in order to construct a pathway of events that leads to the formation of these misoriented divisions.

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P158

Characterization of vegetative and reproductive defects in the maize tassel-less 4 mutant

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Zea mays (maize) is important both as an agricultural crop and as a genetic model organism. Maize normally produces a male reproductive structure called a tassel and female reproductive structure called an ear. However, tassel-less (tls) mutants are characterized by an absent or reduced tassel. At least eight tls loci have been identified, and two have been cloned. Here we present the phenotypic characterization and genetic mapping of the tls4 mutant. tls4 mutants either produce a reduced tassel or no tassel at all. Quantification of tassel phenotypes shows that tls4 mutants produce shorter branches and fewer spikelets (short branches that produce the florets) than their normal siblings, suggesting that the tls4 gene may be involved in the initiation of reproductive structures. SEM analysis of immature tassels shows defects in spikelet pair formation. In addition, tls4 mutants exhibit a number of vegetative phenotypes. First, tls4 mutants are shorter than normal siblings due to shorter stem internode length. Secondly, tls4 mutants exhibit a progressive leaf phenotype with narrow and rough leaves that worsen as the plant matures. Lastly, the leaves of tls4 mutant plants display vasculature defects. Collectively, these phenotypes indicate that tls4 functions in the meristems which are, the pools of stem cells which underlie all growth in plants. Rough mapping using molecular markers indicates that tls4 maps to a 600kb region in bin 10 on chromosome 4. Further fine mapping is ongoing to identify the gene mutated in tls4 plants. We propose that the tls4 gene plays a fundamental role in vegetative and reproductive development in maize.

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P159

Characterize genetic interaction between *fuzzy tassel (fzt)* and *knotted1 (kn1)* in maize

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The maize *fuzzy tassel (fzt)* mutant contains a mutation in a *dicer-like 1* homolog and displays severe defects in both vegetative and reproductive development. *dicer-like1* encodes a critical enzyme for microRNA (miRNA) biogenesis. miRNAs are small non-coding RNAs that play important roles in plant meristem maintenance-homeostasis, determinacy and function. Among other defects, fzt mutant inflorescences are fasciated and multiple meristems are indeterminate. *knotted 1 (kn1)* encodes a homeobox transcription factor that is a master regulator of meristem maintenance. In restrictive genetic backgrounds, loss-of- function *kn1* mutants have a limited shoot phenotype in which the shoot apical meristem is not maintained. In permissive genetic backgrounds however, *kn1* mutant defects are limited to the inflorescence, which have increased meristem determinacy. To investigate the genetic relationship between *fzt* and *kn1* in the inflorescence, we generated a *kn1; fzt* double mutant in a permissive genetic background that does not normally affect vegetative growth of *kn1* single mutants. Surprisingly, the double mutant rarely geminates or arrests soon after germination, suggesting the genetic interaction between kn1 and fzt causes early lethality. We are currently characterizing the cause of this lethality and focusing on the search for possible embryonic and early shoot defects in the double mutant. We are also examining common target genes of miRNAs and KN1 to investigate the molecular underpinings of the early lethality in the double mutant.

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P160

Control of maize plant architecture via Brassinosteroid signaling

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Brassinosteroid (BR) hormones are involved in many aspects of plant development, including promoting growth. To examine BR signaling's role in maize development, we suppressed two members of the BR signaling pathway, BRI1 and BIN2, by RNAi. BLAST searches identified three BRI1-like genes (BRL) and two closely related BRI1 homologs in maize. One of the *bri1* homologs located on chr5 was incomplete in databases, but via subsequent cloning and sequencing was found to be a complete gene. These two closely related *bri1* homologs, named *bri1a* and *bri1b*, share 93% amino acid identity and 95% similarity. *bri1*-RNAi plants had phenotypes including strong dwarf stature and altered leaf morphology. There was also a blurred blade /sheath boundary in these lines. Accumulation of BES1: YFP, a BR responsive marker, in the developing ligule/auricle region suggests that BR signaling is involved in auricle development. For BIN2, a GSK3-like kinase, BLAST searches identified 10 homologs in maize. A phylogenetic analyses by NJ method divided BIN2 homologs into four clades with 5 members belonging to "Clade II", whose members perform primary BR signaling functions in Arabidopsis. All these members had SIWID domain, which is unique to Clade II. *bin2*-RNAi plants had shorter stature, which was unexpected because BIN2 is a negative regulator of BR signaling. However tassel internodes and leaves were longer as expected for increased BR signaling. In contrast to *bri1*-RNAi plants, *bin2*-RNAi plants had larger auricles. The *bin2*-RNAi transgene rescued the mild *bri1*-RNAi phenotype consistent with the predicted position of BIN2 downstream of BRI1 in the signaling pathway. A kinematic analysis on leaf #4 of both *bri1*-RNAi and *bin2*-RNAi showed that leaf elongation rate (LER) was increased 14% in *bin2*-RNAi lines, whereas decreased 17% in *bri1*-RNAi lines. Both cell division and cell elongation contributed to the effects on leaf length in each line.

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P161

Cytokinin can reprogram cellular identity in developing leaves

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The maize leaf has a defined proximal-distal (P-D) growth pattern characterized by four domains with specific cellular identities. The proximal domain is the sheath, the distal domain is the blade, and they are separated by the ligule and auricle. Our analysis of the semi-dominant, gain-of-function, leaf-patterning mutant *Hairy Sheath Frayed1* (*Hsf1*) indicates signaling of the plant hormone cytokinin (CK) can disrupt the P-D pattern. Plants heterozygous for *Hsf1* have outgrowths of tissue with proximal identity, called prongs, arising from the distal blade margin along with excessive macrohairs and smaller, narrower leaves. The *Hsf1* mutant is caused by a missense mutation in the cytokinin receptor *Zea mays Histidine Kinase1* (*ZmHK1*). Our analysis suggests the mutant receptors are CK hypersignaling. To test this idea, we treated B73 seeds with exogenous CK. The resulting seedlings phenocopied many aspects of the *Hsf1* phenotype, including the production of excess macrohairs and prongs. We also determined that *Hsf1* seedlings are hypersensitive to CK treatments. Moreover, treatment of sorghum and rice showed many of these developmental effects were not limited to just maize. We will present detailed results of these treatments and related analysis of cytokinin effects on leaf development.

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P162

Deciphering gene regulatory networks controlling cell differentiation in maize endosperm

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Seed development is initiated by double fertilization of the haploid egg cell and the dikaryotic central cell to produce two filial structures, a diploid embryo and a triploid endosperm, respectively. Endosperm functions as an absorptive structure that supports embryo development or seedling germination in angiosperms. The endosperm of cereal grains occupies a large portion of the mature seed, holds large amounts of proteins and carbohydrates required for seedling development, and is an important source of food, feed, and renewable industrial materials. However, the gene regulatory networks (GRNs) that control endosperm cell differentiation remain largely unclear. As a first step toward characterizing these networks, we used a coupled laser-capture microdissection (LCM) and RNA sequencing (RNA-Seq) strategy to comprehensively profile the mRNA populations present the main cell types of the maize endosperm at 8 DAP, including aleurone (AL), basal endosperm transfer layer (BETL), embryo-surrounding region (ESR), central starchy endosperm (CSE), and conducting zone (CZ). We also captured the embryo and four maternal compartments. We identified mRNAs that specifically accumulate in each of the captured compartments. Also, using an unbiased network analysis tool, we detected modules of co-expressed genes that are either predominantly expressed in a single compartment or highly expressed in multiple compartments. By focusing on a BETL-correlated co-expression module, we identified and experimentally validated a sub-network of the GRN that is activated by MRP-1, a regulator of BETL differentiation and function. These results provide a high-resolution atlas of gene activity in the compartments of the maize kernel and help to uncover the regulatory modules associated with the differentiation of the major endosperm cell types.

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P163

dek34-Dsg1 is a putative *Tel2-interacting protein 2 (Tti2)* important for maize development

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We have characterized a new defective kernel mutant called *dek34-Dsg1*, which we identified from a *Ds-GFP (Dsg)* transposon insertion collection. The *dek34-Dsg1* is a recessive lethal mutant with a *Dsg* insertion in gene model GRMZM2G048851 located on maize chromosome 5. Seed filling and endosperm development stop at approximately eight days after pollination (DAP), resulting in a collapsed seed with reduced starch and protein. Protein bodies are severely affected and appear significantly smaller than those in normal seed, resulting in a reduction of most zein seed proteins. Histological analysis shows that the endosperm cells look mostly identical in shape and size, indicating a failure of cellular differentiation and thus absence of compartments like the basal endosperm transfer layer (BETL). The mutation also causes embryo development to arrest very early at about the pro-embryo stage. Analysis of the predicted protein product of GRMZM2G048851 shows that it contains an Armadillo (ARM) domain, as well as a domain of unknown function (DUF2454). A search in sequence databases shows that this gene is highly similar to human *Tel2-interacting protein 2 (Tti2)*, and is evolutionarily conserved across all organisms. *Tti2* has been found in previous studies to be a component of the TTT-complex, which regulates phosphoinositide-3-kinase-related protein kinases (PIKKs), which in turn play diverse and very important roles in organismal development.

P164

dicer-like3 is required for paramutation, small RNA biogenesis, and normal development in *Zea mays*

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Paramutation refers to a meiotically heritable epigenetic change in gene regulation influenced by *trans*-homolog interactions. These interactions are potentially mediated by small RNAs (sRNAs) as part of an RNA-directed DNA methylation pathway (RdDM). In *Zea mays* (maize), paramutation is observed among specific alleles of the *purple plant1* (*pl1*) and *booster1* (*b1*) loci, both of which encode transcription factors for anthocyanin production. Paramutation can be affected by mutations in genes encoding presumed RdDM components, including the largest subunit of RNA Polymerase IV. A genetic screen for factors *r*equired to *m*aintain *r*epression of a paramutant *pl1* allele uncovered four independent EMS-induced mutant alleles defining the *rmr5* locus. Genetic tests show *rmr5* function is also required to establish repressed states at the *b1* locus. Fine-scale mapping uncovered a candidate gene model encoding a dicer-like3 (DCL3) protein having a likely role in RdDM. In plants, specific DCL proteins process distinctly sized sRNAs from double-stranded RNA precursors. Through sequencing this *dcl3* candidate gene, we identified single transition-type lesions predicting protein dysfunction in all four mutant *rmr5* alleles. We also found sRNA profiles of *rmr5* mutants depleted of 24 nucleotide (nt) RNAs, strongly indicating that *rmr5* encodes DCL3. Unlike in the eudicot *Arabidopsis thaliana*, DCL3 is required for normal maize growth and development, which is consistent with a potentially broader role for RdDM in the grasses. Curiously, these defects are distinct from those reported from other RdDM-type mutants. Additionally, a comparison of 5-methylcytosine (5meC) profiles at a known maize RdDM target shows that high levels of 5meC remain in the absence of DCL3 and that alternatively sized sRNAs occupy previous 24nt positions. These findings point to a specific role of the 24nt RNAs in effecting paramutations and proper maize development.

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P165

Dynamic transcriptome landscape of maize seed

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Maize is one of the most important crops in the world and serves as an excellent cereal model for research on seed development due to its relatively large size for both embryo and endosperm. Despite the importance of seed in agriculture, the genome-wide transcriptome pattern throughout seed development has not been well characterized. Using RNA-seq, we developed a spatio-temporal transcriptome atlas of B73 maize seed development based on 53 samples from fertilization to maturity for embryo, endosperm, and whole seed tissues. A total of 26,105 genes were found to be involved in programming seed development, including 1,614 transcription factors. Global comparisons of gene expression well demonstrated the phases of development. Coexpression analysis provided further insight into the dynamic reprogramming of the transcriptome by revealing functional transitions during maturation. Combined with the published non-seed RNA-seq data, we identified 91 seed-specific transcription factors and 1,167 other seed-specific genes. These seed-specific genes we identified will help elucidate key mechanisms and regulatory networks that underlie seed development. This study provides a valuable resource for understanding the genetic control of seed development of monocotyledon plants.

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P166

Effect of genetic transformation procedure on callus tissues of different maize genotypes

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Genetic alteration of calli of Ukrainian maize genotypes was fulfilled by means of biolistic and *Agrobacterium*-mediated transformation with constructions included alien genes *npt*II, *uid*A and *bar*. Callus screening was made on selective media supplemented with phosphynothricine (10 mg/l) for *bar* selection and paromomycine (100 mg/l) for *npt*II selection. Transient expression of *uid*A gene has been successfully confirmed histochemically, but the occurrence of *bar* gene in calli and regenerated plants has been verified by PCR. The rate of decrease of morphogenic callus formation depended on transformation technique, selective medium composition, inbred or hybrid genotype of initial explant. Under genetic transformation and further cultivation on corresponded selective media during 30 days the common level of morphogenic callusogenesis decreased in comparison with control variant less (by 27.2-33.1%) than for intact calli (by 36.5-41.1%). A positive effect of genetic transformation was manifested remotely on the 60th day of cultivation (30 days on induction medium and 30 days on regeneration one). The level of plant regeneration under selective pressure for genetically modified calli composed 3.1-28.9% while in non-treated the process of plant regeneration was blocked up entirely by selective agents. After genetic transformation inbred PLS61 and hybrid DK267×PLS61 have appeared the most capable to morphogenic callusogenesis and regeneration under selective pressure. Hybrid of popcorn maize PRG5×KP7 had the least ability to callusogenesis as in control, so as after transformation and further cultivation on selective media. In general, callus tissues of type I (compact) and type II (friable) of Ukrainian hybrids of different maize germplasms and subspecies are sensitive to genetic transformation.

P167

Evolution of inflorescence development in maize and related grasses

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Meristems control organogenesis in plants through the maintenance of groups of undifferentiated stem cells. Upon completion of vegetative development, the shoot apical meristem is converted into the inflorescence meristem. Inflorescence meristems of grass species (Poaceae) give rise to spikelets, which house the male and female floral organs and are the fundamental units of grass inflorescence architecture. Although solitary spikelets are the ancestral trait shared by several major agro-economic crops (i.e., wheat and rice), paired spikelets are produced by species in at least three Poaceae tribes (i.e., Paniceae, Paspaleae, and Andropogoneae), including maize. At least three loci in the *Suppressor of sessile spikelet (Sos)* class of mutants have been identified which regulate the production of paired spikelets in maize. *Sos1, Sos2,* and *Sos3* prevent the formation of the sessile spikelet, causing only the ancestral single spikelet to form. Each of these mutants also have characteristic growth defects indicating additional roles in inflorescence development. Identification of the *Sos* genes will further our understanding of the evolution of the derived paired spikelet trait. Additionally, Mesquite ancestral character state reconstruction is being used to elucidate the number of times the paired spikelet trait arose in the evolution of the grasses. We plan to use comparative expression and synteny analyses of *Sos* and related inflorescence development genes, together with developmental analyses, to investigate the differences between paired and solitary spikelet development in selected grass species.

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P168

Exploring the potential role of the INDETERMINATE DOMAIN members of transcription factors in the specification of bundle sheath and mesophyll cells identity

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Compartmentalization of photosynthesis into two cell types in C4 species is an important event in evolution as it allows more efficient carbon fixation under dry, hot conditions and consequently, an increase in grain and biomass yield. Nonetheless, the signals that drive the structural alterations in C4 leaves are yet to be unraveled. It has been hypothesized that a regulatory network involving the transcriptional regulation of SCARECROW/SHORT-ROOT (SCR/SHR) and the INDETERMINATE DOMAIN (IDD) family of transcription factors acts to determine cell identity in the leaves of C4 species (Slewinski et al, 2013). To explore the potential function of IDD members in bundle sheath/mesophyll (BS/M) cell specification we retrieved all sequences from Maize, Rice, Sorghum, Setaria and Arabidopsis and performed a phylogenetic analysis to determine putative functional orthologs. Subsequently, we analyzed leaf gradient transcriptomic datasets to identify members that are co-expressed in the leaves of Maize, Setaria, Sorghum and Rice. In Maize, out of the 23 members, 12 genes are expressed in the leaves; in Setaria, eight genes out of 15; Rice, nine genes out of 15; and Sorghum, eight out of 17. Additionally, cell-specific expression profile was determined for the leaf-expressed IDD members in Maize, Setaria and Sorghum using available BS/M datasets (unpublished). Four members of the IDDs are co-expressed in the leaf gradient and are enriched to the mesophyll cells. Loss of function analyses of these candidates are being evaluated using available *Ac/Ds* and *Mutator* populations and by RNAi in *Setaria viridis*. Co-expression analyses allowed us to identify members of the IDD family that may play an important role in the specification of photosynthetic cells identity in C4 species.

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P169

Fine mapping of a major locus regulating the transition from juvenile to adult phase in maize

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Maize goes through three main developmental phases, namely juvenile vegetative, adult vegetative, and reproductive. The juvenile vegetative phase is characterized by leaves with epicuticular wax, no epidermal hairs and thin cuticle. The transition to adult phase (disappearance of epicuticular wax, and development of hairs and thick cuticle) takes place at leaf 6-7, although it is genotype-dependent. In this work we report the fine mapping of a locus governing the juvenile-to-adult transition in maize. A B73-Near Isogenic line (NIL) from a Gaspé Flint/B73 introgression library (Salvi et al. 2011. BMC Plant Biology) showed a prolonged juvenile phase (transition at approx. leaf 9, compared to leaf 6-7 of B73). The B73-NIL carries an introgression of approximately 35 Mb from Gaspé Flint in bin 3.05, between markers bnlg156 and umc2265, based on high-density SNP genotyping. A B73 x B73-NIL F2 population of >2,000 plants was scored by marking the leaf showing transition between juvenile and adult phase. Analysis of trait inheritance showed that early (B73-like) transition was incompletely dominant over late (Gaspé Flint-like) transition. Fine mapping is being carried out using SSR and SNP markers. The chromosome region targeted in this study has been already shown to include QTLs for developmental phase transition (Foerster et al. 2015, TAG) and encompasses several candidate genes, including the locus for miR172, previously reported to regulate the AP2-like gene glossy15, involved in promoting juvenile leaf traits (Lauter et al. 2005, PNAS).

P170

Fine mapping of the maize *dosage-effect defective kernel*-30* (*ded*-30*) locus.

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Grain yield and seed weight are important target traits for the improvement of seed crops. To identify genes that control maize seed weight, we screened 1,000 *defective kernel* mutations from the UniformMu transposon-tagging population for seed dosage-effects using individual seed weight and single-kernel near infrared reflectance spectroscopy. The *ded*-30* mutant was identified in this screen as showing segregation distortion when normal kernels are separated by seed weight. Lower weight, normal seeds showed an enrichment for *ded*-30*/+ plants. Homozygous *ded*-30* mutant seeds are visibly distinct from normal at 10 days after pollination. Mature homozygous mutant seeds have severely reduced grain-fill and are defective in both endosperm and embryo development. The *ded*-30* locus was mapped to the long arm of chromosome 1 using bulked segregant analysis of F2 mapping populations from crosses of *ded*-30* allele with B73 and Mo17 inbred lines. Fine-mapping identified a 358 kb interval that contains the *ded*-30* locus. The mapping interval contains 22 annotated gene models. One of these genes is a predicted transcription factor and shows a polymorphism between the W22 genome and the *ded*-30* allele. DNA sequencing of this gene revealed that it contains a retrotransposon insertion in the 3' region of the open reading frame. These data suggest that the transcription factor gene is likely the *ded*-30* locus.

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P171

Florigen-encoding loci of autonomously flowering and photoperiod-sensitive maize are associated with different chromatin modifications at the floral transition

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Activity of the maize florigen gene Zea CENTRORADIALIS 8 (ZCN8) is associated with the floral transition in both day-neutral temperate maize and short-day (SD) requiring tropical maize. We analyzed transcription and chromatin modifications at the ZCN8 locus during floral transition, along with its nearly identical paralog and putative additional florigen encoding gene ZCN7. This analysis was performed with day-neutral maize (Zea mays spp. mays), where flowering is promoted almost exclusively via the autonomous pathway through the activity of the regulatory gene indeterminate 1 (id1), and tropical teosinte (Zea mays spp. parviglumis) under floral-inductive and non-inductive photoperiods. Comparison of ZCN7/ZCN8 histone modification profiles in immature leaves of non-flowering id1 mutants and teosinte grown under floral inhibitory photoperiods reveals that both ID1 floral inductive activity and SD-mediated induction result in histone modification patterns that are compatible with the formation of transcriptionally competent chromatin environments. These epigenetic signatures are established during leaf development and may facilitate the production of processed ZCN7/ZCN8 mRNA in florigen-producing mature leaf. However, whereas ID1 functional promotes histone H3 hyper-acetylation, SD induction is associated with increased histone H3 di- and tri-methylation at lysine 4. This suggests that distinct mechanisms distinguish florigen gene regulation in response to autonomous and photoperiod inductive pathways.

Funding acknowledgement: Natural Sciences and Engineering Research Council of Canada, Epigenomics Flagship Project (EPIGEN), National Research Council of Italy

P172

Function of KNOX cofactors, the BEL1-like homeobox proteins in maize shoot meristems

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The shoot meristem is an indeterminate structure responsible for the formation of all above ground organs in flowering plants. The *knotted1*-like homeobox (*KNOX*) family genes are essential for the establishment and maintenance of the shoot meristems. Chromatin immunoprecipitation followed by sequencing study in our laboratory suggested that maize KNOTTED1 (KN1) has more than 5,000 putative targets, however, the molecular mechanism how it achieves their transcriptional regulation remains elusive. In *Arabidopsis*, BEL1-like homeobox (BLH) proteins have been shown to interact with KNOX proteins. We thus focused on two maize BLH proteins, BLH12 and BLH14, putative protein partners of KN1. Bimolecular fluorescence complementation assay showed that both BLH12 and BLH14 interact with KN1. Immunostaining showed that both BLH12 and BLH14 proteins accumulate in a pattern overlapping with KN1. Although each single mutant showed normal development, *blh12 blh14* double mutants had various developmental defects including reduced internode length and lack of tillers and ears. In the double mutant tassels, spikelet meristem initiation failed, and instead branch meristem-like structures were repetitively formed. These results suggested that BLH12 and BLH14 are important protein cofactors for KN1 in maize shoot meristems.

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P173

Genetic Analysis and Positional Cloning of the *Few-branched1* and *Unbranched** Mutations Involved in Bract Suppression in Maize

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Bracts are lateral organs that subtend a flowering branch or a flower. In some angiosperms bracts are showy and/or brightly colored, while in others they are reduced or completely suppressed. The genetic network of bract suppression is still unclear. In maize (*Zea mays*), bracts are completely suppressed and several mutants including *tassel sheath1* (*tsh1*), *tsh2/4*, *tsh3*, *tsh5*, *Few-branched1* (*Fbr1*), and multiple enhancer of *tsh1* have been identified to affect bract outgrowth, even though only *tsh1* and *tsh2/4* have been functionally characterized. The semi-dominant *Fbr1* mutants exhibit reduced or no branching (depending on the genetic background) with ectopic bracts subtending the reduced or suppressed long braches in the tassel. At early stages of tassel development, mutant meristems of *Fbr1* initiate excessive bract primordia accompanied by the abortion of their axillary long branches even though some bract primordia fail to further develop. We mapped the *Fbr1* gene to an approximately 88 kbp segment on the long arm of chromosome 6 containing one candidate gene, a putative transcription factor. A putative second allele of *Fbr1*, the dominant *Unbranched** (*Ub**) was also mapped to the same region within a 15.8 cM interval. Both *Fbr1* and *Ub** mutants have the same conservative missense point-mutation, a A to V substitution which is absent from the prospective progenitors. How this point mutation might disrupt or alter the normal protein function is under investigation. Putative loss of function mutants have been isolated and are also under investigation.

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P174

Genetic enhancers of *Hairy Sheath Frayed1* leaf patterning defects

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The maize leaf is composed of four compartments—sheath, ligule, auricle and blade—polarized along the proximal-distal (P-D) axis. The *Hairy Sheath Frayed1* (*Hsf1*) mutant has an altered P-D patterning phenotype, characterized by outgrowths of proximal tissue from the margin of the distal leaf blade. This phenotype is caused by missense mutations in *Zea mays Histidine Kinase1* (*ZmHK1*), a receptor for the growth hormone cytokinin. These mutations result in cytokinin hypersignaling of the receptor, leading to an altered P-D pattern. Misexpression of specific class I *knotted-like homeobox* (*knox*) genes are also known to alter P-D patterning by disrupting the blade-sheath boundary. Many *knox* genes have also been shown to directly control the accumulation of CK. To test the epistatic interaction between *Hsf1* and *knox* gain-of-function mutants, the leaf morphology of double mutants was analyzed. We found certain *knox* misexpression mutants enhanced the *Hsf1* phenotype in specific ways. For example, the *Liguleless3* (*Lg3-O*) mutant caused more *Hsf1* blade margin to be converted to proximal tissue. This is consistent with *lg3* being the only class I *knox* gene misexpressed in developing prongs. We will present additional interaction data, focusing on specific enhancements of patterning defects in *Hsf1*.

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P175

Genome-Scale Nitrogen Responsive Gene Expression during Maize Development

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Nitrogen (N) is a key limiting plant nutrient and its availability is expected to have significant impacts on the expression of genes that function in nitrogen metabolism and growth responses to N. Although a key target for improving maize yield response to nitrogen, relatively little is known about the gene regulatory systems that modulate N remobilization. By profiling N remobilization can be explored as the plant senesces and moves nutrients from the leaf to the developing ear or grain fill of the seed. Classes of genes that show coordinated transcriptional responses across different tissues and developmental stages may indicate key control points in N cycling between source and sink tissues. Of particular interest are the 170 genes which respond to N in opposite directions between the leaf and ear tissue at the same developmental time point. In addition, a curated set of genes known to function in N metabolism were used to validate expected expression patterns and identify the specific N responses within these pathways. In total 10,847 of the 29,933 expressed genes were differentially expressed in response to N in at least one tissue; in any one sample between 3% and 10% of the reads were differentially expressed. Our analysis shows the N response is context dependent; N responsiveness cannot be evaluated globally or by taking a snapshot of expression data as previous studies have done. The developmental context informs the N response and should be considered in future breeding for improved N remobilization efficiency. N remobilization genes may need specialized promoters to confer fine-tuned regulation in the correct developmental context, or we may need to identify the genes which are tipping points to the balanced nitrogen system as a strategy for improvement.

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P176

Grass-specific diSUMO-like DSUL interacts with substrates differing from SUMO substrates to control the first zygotic division in maize

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Dynamic SUMO (small ubiquitin-like modifier) modification plays essential roles in regulating chromosome assembly and cell cycle progression in eukaryotes. The molecular mechanism and the specific role of different SUMO variants during meiotic and mitotic cell division processes are almost unexplored in plants. We report that a grass-specific diSUMO-like protein, DSUL, is exclusively detected in the egg apparatus before fertilization and most abundant in the zygote before asymmetric cell division occurs. RNAi silencing studies show that DSUL is required for nuclei segregation and positioning during zygotic and first embryonic cell divisions in maize. DSUL activity and subcellular localization are associated with cell division and are strongly different from monoSUMO (here SUMO1b expressed both in egg cells and zygotes was used as an example). In accordance with these observations, proteomic analyses of DSULylated proteins identified a distinct set of DSUL substrates, including those involved in chromosome segregation, spindle length regulation and cell cycle progression, which are significantly different from SUMO1b substrates. These findings indicate that DSUL controls asymmetric zygotic division through the specific modification of key components of the mitotic division machinery. Taken together, we propose that unlike SUMOylation, which represents a mechanism for rapid and global manipulation of chromosomal functions, DSULylation plays an important role in nuclei positioning during asymmetric cell division in the grasses. Whether diSUMO plays a similar role in eudicots remains to be shown in further experimentation.

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P177

Heterotrimeric G protein signaling in maize shoot development

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Heterotrimeric G proteins are membrane-associated molecular switches involved in the transduction of extra cellular signals to induce specific cellular responses by activating downstream effectors. They are composed of the three subunits, Gα, -β and -γ, and are normally activated via binding of an extra-cellular ligand to a 7-pass trans-membrane G protein-coupled receptor (GPCR). GPCRs cause Gα to exchange GDP for GTP, leading to the dissociation of the complex into Gα-GTP and Gβγ dimer. These subunits activate different signaling cascades, until the GTPase activity of Gα hydrolyzes the GTP molecule, leading to the re-association of the inactive heterotrimer.
Although plant cells have most of the core elements found in animals, research of the past 10 years revealed that G protein signaling in plants is fundamentally different. Whereas animal genomes have many different heterotrimeric subunits, most plants have only one canonical Gα-, one Gβ-, and up to six Gγ-subunits. It has also been realized that G protein signaling in plants does not follow the canonical signaling mechanism established in animal system. We identified *COMPACT PLANT2 (CT2)* as the maize α-subunit of the heterotrimeric G protein. The phenotype of *ct2* mutants is pleiotropic; they are semi-dwarfed and strongly affected in leaf development, as the mutants produce short, dark green, and erect leaves. In addition *ct2* shoot meristems are enlarged. We showed that CT2 interacts with FEA2, which belongs to the abundant class of LRR receptors. This observation might have wide-ranging implications for other areas of plant biology such as BR signaling, which is also mediated by LRR receptors. This research plan represents a systematic approach to further explore heterotrimeric G protein function as a central integrator, necessary to synchronize extracellular signal perception, intracellular signal transduction, and cytoskeleton dynamics important for controlled cell proliferation. We will characterize the role of CT2 in internode development to ask if other pathways, such as BR signaling, are integrated with MT dynamics and cell proliferation through heterotrimeric G protein activity. We will also pursue an EMS induced enhancer/suppressor screen and a natural modifier screen of *ct2* using the NAM population. Finally, we will complete our understanding of heterotrimeric G protein following a functional genomics approach to characterize the six maize Gγ-subunits using the CRISPR/Cas9 technology.

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P178

Identification of temporal regulatory modules in early maize endosperm development

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Endosperm is a product of double fertilization and functions as a nutritive tissue in the angiosperm seed to support the growth of the embryo or the germinating seedling. In cereal grains, endosperm comprises a large proportion of the mature seed and contains large amounts of carbohydrates and proteins. To identify a high-resolution temporal transcriptome of the earliest stages of endosperm development, we used laser-capture microdissection to isolate and profile mRNA populations of a developmental series of the endosperm from 0 to 4 DAP in maize inbred B73. These stages comprise the initial period of proliferation of the triploid endosperm coenocyte through cellularized endosperm that shows an overall polarity and indications of early cell differentiation. Using computational tools, we identified distinct temporal co-expression modules during this period of development. Analysis of the co-expressed transcription-factor genes and the associated cis-regulatory elements allowed us to hypothesize gene regulatory networks involved in early endosperm development in maize.

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P179

Inter-compartment communication in maize seed

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Correct maize seed development relies on interaction and communication between three seed compartments: embryo, endosperm and seed coat. Despite genetic evidence pinpointing the importance of this inter-compartmental communication, the underlying molecular mechanisms remain unknown.

This work has the objective to elucidate the molecular signaling frameworks operating between embryo, endosperm and seed coat, using a combination of genome-wide and targeted approaches: (i) Firstly, an integrated analysis of the transcriptome from each dissected compartment and the proteome of the interfaces (cell wall proteome and secretome) is undertaken to identify signaling proteins capable of moving between adjacent compartments. (ii) Secondly, the functional characterization of a class of signaling peptides (CLE peptides) is underway. Three CLE peptides have been shown to be secreted into the embryo surrounding region (ESR) of the endosperm. To investigate the *in vivo* role of these peptides on the ESR and/or embryo, transgenic plants expressing an antagonist CLE peptide under an ESR-specific promoter have been obtained. These plants are being examined for dominant negative phenotypes caused by the modified CLE peptide. In addition, we are using an *in vitro* root assay to test the biological activity of both native and antagonist peptides.

We expect that these complementary approaches will uncover an unprecedented number of interactions underlying seed compartment coordination, providing the basis for an integrated view of how signaling pathways synchronize the development of the three compartments.

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P180

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, 31 driver constructs have been produced to drive expression in shoot and inflorescence meristems, leaves or roots using tissue-specific promoters. Five responder constructs are currently completed and being analyzed, including *Zea mays* FON2-LIKE CLE PROTEIN1 (ZmFCP1) and the FLOWERING LOCUS T like *Zea mays* CENTRORADIALIS 8 (ZCN8). As an experimental approach we are scoring the effect of ectopically expressing these regulatory proteins in different developmental contexts, and recent advances will be presented. Transformants have been obtained for 91% 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. 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)

P181

Maize Transformation Services

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University of Missouri (MU) Plant Transformation Core Facility has been providing state-of-the-art plant transformation services over the past 14 years. The facility is aiming at fostering plant science research by providing transformation services worldwide. The services are on fees for cost recovery only, not for profit. The facility staff is dedicated to providing various types of transformation services. Maize (*Zea mays*) transformation via Agrobacterium-mediated approach is one of our major service categories. Our maize transformation service includes both Hi II maize and inbred B104 transformation. In addition, we also provide transformation services for soybean (*Glycine max*), switchgrass (*Panicum virgatum*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), alfalfa (*Medicago truncatula*), as well as *Setaria viridis*. The service categories include both standard and customized transformation. Transformation systems for all crops utilize Agrobacterium-mediated approaches and somatic embryogenesis processes except for soybean and *Medicago*. The Agrobacterium-mediated cot-node transformation system coupled with organogenesis regime is employed for soybean and *Medicago* transformation. The facility is also ready to take on new service projects to transform new plant species as user's requests. Research activities are geared towards developing high-throughput transformation systems, effective small RNA-mediated gene silencing, gene stacking through coordinated transgene expression, and precise genome modifications to meet the needs of crop improvement and genome discoveries. More details on the facility can be found at http//www.plantsci.missouri.edu/muptcf.

P182

Male Gametophyte-Specific Expression Helps Identify A Conserved Gene Associated with Increased Pollen Fitness

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GRMZM2G372877 was identified as a gene with potential function in the male gametophyte based on its strong expression in mature pollen relative to other maize tissues (Chettoor et al. 2014). Identification of a *Ds* insertion mutation in this gene from the Brutnell/Vollbrecht collection provided further support for this hypothesis, as initial data indicated the insertion was associated with a male-specific transmission defect. In this study, we confirmed the location of *GRMZM2G372877* on chromosome 9, approximately 25 map units away from *wx1*. We used linkage of the *Ds* insertion to *Wx+*, as well as PCR genotyping, to follow up on the initial results, confirming a male-specific transmission defect from mutant heterozygotes. Because the severity of the transmission defect varied with different crosses (2% to 13%), we tested the idea that the defect decreased pollen fitness when in competition with wild-type pollen. Consistent with this idea, we found that male transmission of the mutation increases in frequency when less pollen is applied to the silk (12% to 43%). Based on DNA sequence, we found *GRMZM2G372877* was orthologous to a gene (*delegen14*) included in a 65-kb deletion associated with the rice no-pollen mutant (*Osnop*) (Jiang et al 2005), suggesting a conserved function for this gene in pollen. We have tentatively named the gene *nop1*, and it encodes a protein with C2 and GRAM domains that are predicted to interact with calcium and phosphoinositides, respectively. Results from microscopy experiments, to visualize specific cellular defects, and to help better determine the function for this gene in pollen or pollen tube development, will also be presented.

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P183

Mapping and Characterizing the Maize Mutant *Clumped tassel1* and its Modifier Locus *mcl1*

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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 fine-mapped *Clt1* to a 530kb region on Chr 8 containing only 16 predicted genes and used NextGen sequencing to identify one of these genes (GRMZM2G017305), predicted to encode the microtubule-severing protein katanin, as a candidate. We are verifying that we have cloned the causative mutation by transforming the mutant and wildtype alleles of maize katanin into *Arabidopsis lue1* mutants, which have defective native katanin p60, hypothesizing that only the wildtype maize allele will complement the mutant phenotype.

To further understand *Clt1* function, we crossed *Clt1* (B73) with the 27 NAM founder lines to screen for naturally-occurring modifier genes. In the F2 of a cross between *Clt1* (B73) and Ki11, we observed segregation of a novel *Clt1* phenotype where the internodes in the upper parts of the plants were dramatically compressed. Using bulked segregant analysis (BSA), we mapped a modifier locus that we have named *mcl1*, to a ~20Mb interval on Chr 3. We have confirmed a strong epistatic relationship between the *Clt1* mutant allele and the Ki11 allele of *mcl1* by genotyping and phenotyping individual F2 plants. Interestingly, this Chr 3 region is homoeologous to the chr 8 region containing *Clt1* and contains a duplicate gene of katanin p60 (GRMZM2G054715). Public sequencing data indicates that the Ki11 allele of GRMZM2G054715 includes a premature stop codon, suggesting its function is compromised, and public RNAseq data revealed that Ki11 has low expression of the *Mcl1* katanin. Interestingly, NAM founders with similar,low expression of *Mcl1* katanin also segregate plants with compressed upper internodes when crossed to *Clt1*, but of the lines we measured,those with high expression do not.

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P184

Morphology and Characterization of Abscission Zone Development and its Role in Domestication in *Setaria viridis* and *Setaria italica*

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Reduced shattering or the increased retention of seeds on plants beyond senescence is one of the key traits that has allowed for the domestication of cereals (Poaceae). Selection on this trait alters the functionality of the abscission zone that seeds utilize for release at maturity. Despite the importance of shattering, abscission in cereals remains poorly understood in systems other than rice. To alleviate this lack of resolution a new system, *Setaria viridis*, was selected to identify the underlying patterns related to abscission zone development within the previously uncharacterized tribe Paniceae (Panicoideae). For this study one line of the wild species *S. viridis* and two lines of the domesticated *S. italica* were used. Tensile strength measurements revealed not only that *S. italica* is more resistant to shattering than *S. viridis* but also that the manner in which shattering is reduced differs drastically from what is observed in rice. Anatomical differences were also noted in which the transverse cell layers of the *S. viridis* abscission zone are far more distinct than in either accession of *S. italica*. In addition, *S. viridis* retains viable cells late into maturity whereas corresponding cells prematurely senesce in *S. italica*. Gene expression studies were also conducted on known shattering genes from other systems throughout the flowering time of both *S. viridis* and *S. italica* to see if these genes have retained expression within this genus. The gross differences in abscission zone cellular patterning and position within the spikelet axis between *S. viridis* and rice suggest that many features related to loss of shattering in *S. italica* are unique to this lineage.

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P185

Morphometric comparison of maize endosperm and nucellus development in B73 and diverse NAM founder lines

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Early maize endosperm development occurs in four cytologically identifiable stages: coenocytic, cellularization through alveolation, cellularization through partitioning, and differentiation. Onset of cellularization is coincident with endosperm size during the initial days after pollination (DAP) in the reference inbred B73. We hypothesized mature kernel size may correspond to endosperm or nucellus size during early development. We used mature kernel phenotype data from Panzea to choose Nested Association Mapping (NAM) founder lines with relatively small (Hp301, P39, NC350, NC358) or large (Ky21, M162W, B97) kernels. Length, thickness, and area of kernel compartments were measured using medial longitudinal sections from field-grown kernels (0-6 DAP) collected in 2011. Differences between the lines in terms of the proportion of the kernel occupied by endosperm related to mature kernel size, and were most apparent at 4-6 DAP where B73 was intermediate to the fastest (Hp301, P39) and slowest (Ky21, M162W) lines. In order to evaluate endosperm size during all four stages of early development, we further analyzed these lines from 0-12 DAP using field-grown kernels collected in 2013. We found that large kernel lines had larger and more persistent nucellus tissues, suggesting that kernel size in early development is largely influenced by maternal contribution through nucellus size, rather than endosperm. The endosperms of large kernel lines consistently occupied a smaller portion of the caryopsis from 3-12 DAP. Cytological examination showed that small kernel lines not only have endosperms that more quickly occupy the kernel, but also initiate and complete cellularization faster than large kernel lines.

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P186

Mutations identifying *required to maintain repression12* affect development and paramutation

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Paramutations result in heritable changes in gene expression and are influenced by poorly understood *trans* homolog interactions (THI). Mutational analyses implicate a potential RNA directed DNA methylation (RdDM) mechanism in mediating this THI. In *Zea mays*, paramutation occurs among specific alleles of the *purple plant1* (*pl1*) locus that encode MYB-type transcription factors necessary for anthocyanin pigment production. A genetic screen for factors *r*equired to *m*aintain *r*epression of paramutant *pl1* alleles identified ethylmethane sulfonate-induced mutant alleles of a novel locus, *rmr12*. Homozygous *rmr12* mutants display transmission and developmental defects not seen with other RdDM-type mutants described so far thus implicating a novel epigenetic suppression function. Transmission frequencies for all three mutant alleles are less than expected of single locus recessive mutations but the reason for this transmission ratio distortion is currently unknown. Homozygous *rmr12* mutants used as pistillate parents show variable kernel abortions. These observations indicate a sporophytic defect in supporting proper ovule or post-fertilization development. Measurements show *rmr12* mutants have abnormal leaf development and growth defects strongly affecting height and flowering. Preliminary results indicate *rmr12* function affects 24nt RNA abundances, which is consistent with other *rmr* mutations that identify an RdDM-type mechanism. These results further expand the role of 24nt RNAs in controlling maize ontogenesis in contrast to *Arabidopsis* in which RdDM mutants have little effect on development.

Funding acknowledgement: United States Department of Agriculture (USDA)

P187

narrow odd dwarf, a maize developmental mutant

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The establishment of plant architecture is a complex process that relies on the function of multiple genes, acting in various developmental pathways. The shoot apical meristem (SAM) is responsible for the production of above ground organs and, therefore, regulation of its activity is required for correct plant development. We have identified *nod* (*narrow odd dwarf*), a recessive maize mutant with severe pleiotropic developmental phenotypes. NOD is the maize ortholog of the plant-specific MCA (mid-complementing activity) protein. MCAs are broadly expressed membrane-localized proteins with suggested calcium channel-related activity. They function in development, mechanosensing and calcium absorption in roots in Arabidopsis and rice. Severe developmental defects have only been observed in maize. *nod* mutants have an overall reduction in plant size, due to lower height and narrow, short leaves. Additionally, loss of apical dominance in B73 leads to the presence of multiple tillers that give plants a small bushy appearance. Proximal distal patterning is also affected. The maize leave is normally composed of a proximal sheath and distal blade with the fringe-like ligule and auricle forming a clear border between sheath and blade. In *nod* leaves, the border between sheath and blade is abnormal and ligules are not correctly positioned. This results in the presence of both sheath and auricle cells in the blade portion of leaves. Defects get progressively worse with SAM function. *nod* plants are affected in various other aspects of development including, juvenile to adult transition, stomatal patterning and inflorescence branching. This suggests a multi-faceted role of NOD in maize growth and developmental processes.

P188

Natural variation and drought responses in developing maize inflorescences

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Most drought stress studies in maize have focused on mid and late season growth periods, however early season drought stress, which affects the establishment of ear development programs mediated by meristem activities, may also lead to substantially reduced yields. Predicted changes in global climate and expanded cultivation of maize in developing countries will likely increase the impact of early season stress on yields. Thus, understanding the genes and gene interactions that control maize inflorescence development, how this transcriptional network responds to abiotic stress and how it is modulated by natural variation, may provide valuable agricultural tools. In collaboration with DuPont-Pioneer, we have established and replicated drought treatments in a greenhouse environment to impose a quantified stress during ear intitation and early development. Consequences of stress treatments are being characterized by spatial and temporal phenotyping and gene expression profiling. Under drought stress, ears cease growth and exhibit developmental defects including disturbed patterns of rows. Upon rewatering, normal row patterns are re-established, suggesting developmental plasticity of inflorescence meristem programs. In initial high-throughput profiling experiments using selected developmental and stress response genes, hierarchical clustering indicates similar expression profiles for well watered controls and rewatered, previously stressed ears, consistent with the morphological data. Developmental and stress genes are differentially regulated, and we observe a significant interaction between the water regime and the developmental stage at the time of stress. Large-scale, RNA-seq experiments are now underway, to ultimately resolve with which regulatory mechanisms in the heirarchy controlling inflorescence development, drought stress interacts. This project cultivates broad and comprehensive training opportunities across diverse disciplines that encompass plant development, quantitative genetics, abiotic stress responses and bioinformatics, including through workshop activities to serve several educational levels.

Project web site: http://www.maizeinflorescence.org

Funding acknowledgement: National Science Foundation (NSF)

P189

Over-Expression of the Photoperiod Regulator ZmCCT10 Resulted in Apically-Induced Plantlet Formation on Transgenic Maize Plants

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Maize was domesticated from short day grass teosinte. While tropical maize is photoperiod sensitive, temperate maize is day neutral - in part due to the loss of transcriptional activity of the photoperiod regulator ZmCCT10. To gain insight into pathways regulated by ZmCCT10, a cohort of transgenic events was generated that over-express ZmCCT10 alleles isolated from four germplasms: teosinte, tropical, temperate, and early flowering Gaspé Flint. Variation of transgene expression was created by using two promoters: maize ubiquitin (UBI) and viral BSV, which is several-fold stronger than UBI. Constructs were transformed into Gaspé Flint, which typically produces 7-10 leaves. The strength of the promoter, regardless of allele origin, determined the phenotypes of the T0 transgenic events. UBI transgenic events exhibited a late flowering phenotype with a maximum of 22 leaves, and delayed shedding and silking. Unusually elongated shanks were also observed. Transgenic BSV events displayed the extreme vegetative phenotype of prolonged juvenile development producing up to 57 leaves and adventitious roots on the stalk up to node 37. Multiple reproductive abnormalities were observed including little to no ear initiation and abnormal development of the apical meristem. Instead of a tassel, the apical meristem of BSV transgenic events formed a combination of plantlets and tassel branches, a phenotype which is similar to “crazy top” (Sclerophthora macrospora) infected plants. Apically-induced plantlets may have up to 18 leaves, adventitious roots, and reproductive (ear-like and tassel-like) structures that sometimes produced pollen or set kernels. The defective tassel phenotypes of tassel seed and tassel sheath (glumes reverting to husk-like leaves) were also observed. In some tropical lines, exposure to long days generates apically-induced plantlets which suggest that this phenotype is naturally occurring under certain environmental conditions. ZmCCT10 appeared to be involved in regulation of the complex genetic networks that control transition from juvenile to reproductive development.

Funding acknowledgement: Dupont Pioneer

P190

Parallel approaches towards identification of novel factors regulating tiller growth in maize

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Tillers are an important agronomic trait with effects on grain yield. Tb1 and Gt1 are transcription factors which regulate tiller outgrowth in maize. Whereas most maize inbreds lack any tillers, loss of function for either Tb1 or Gt1 results in multiple tillers. In addition, regulatory changes in both of these genes have been selected during domestication, further highlighting the importance of the mechanisms they regulate. Genetic and expression analysis revealed that Tb1 acts upstream of Gt1. We have initiated a developmental analysis of tiller bud growth dynamics in these mutants compared to wild type. Our results show that the first tiller bud of B73 enters dormancy at about 9 to 12 days after plant (DAP), while the second bud of B73 becomes dormant in about 12 to 15 DAP. However, the buds of the gt1 and tb1 fail to enter a dormant state, and continue elongating. To better understand the mechanisms that control tiller formation, we plan to profile the transcriptome of early stage tiller buds from gt1 mutant, tb1 mutant and wild type B73 by RNA-seq. In addition to transcriptome profiling, we are also screening for interaction partners of the GT1 and TB1 proteins by yeast two hybrids, as well as genetic modifiers. By combining these approaches we hope to identify novel factors regulating tiller development in maize and begin to reveal the genetic network of maize tiller regulation.

Funding acknowledgement: National Science Foundation (NSF)

P191

Phototropism in maize: revisiting classical physiology.

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Plants utilize several classes of light receptors to perceive environmental light cues. These cues are used to mediate adaptive growth changes to maximize photosynthetic light capture and plant success. One specific class of photoreceptors, the phototropins, activate the blue light signaling pathway necessary for responses such as phototropism, stomatal opening, cotyledon and leaf expansion, and root architecture. Phototropism, a growth response to directional light cues, has been used to observe plant movement responses even before Darwin's *The Power of Movement in Plants* . While various crop seedlings, including maize, were popular models for many of the classical physiological studies, the molecular mechanisms underlying phototropism and blue light adaptive responses, have recently been more extensively studied in model organisms such as Arabidopsis. These studies have elucidated many components of the molecular mechanism underlying blue light perception, signaling and response. We aim to determine the signaling components involved in blue light perception and response in maize, with the ultimate goal of understanding their role in developmental changes in response to stress, specifically drought. We are currently utilizing a forward and reverse genetic approach, followed by a classic phototropism assay, to identify putative components of the tropic response pathway in maize. Interestingly, we are able to address questions that would be otherwise difficult in a model organism like Arabidopsis, simply based on the size of the maize seedlings.

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P192

Positional cloning and characterization of a gene required for normal cellular elongation in maize (*Zea mays* L.)

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In maize, *Zea mays*, as in other grass species, cellular elongation occurs during growth. Single gene mutations have been used to develop crop varieties with reduced heights so as to reduce stem breakage, or lodging. Here, we describe two, true breeding, dwarf maize mutants. Both mutants mapped to a ~98 Kb genomic interval, and each maize mutant allele has a missense mutation at a highly conserved amino acid residue within a candidate gene. The cellular morphology of mature, dwarf plants differs from wild type plants in longitudinal sections but appears normal in transverse sections. Cell elongation requires vesicular transport of cell wall components and plasma membrane domain proteins. The thirty-six differentially expressed genes between mutant and wild type plants suggest that mutants are impaired in this vesicular transport. Mutant alleles could be utilized to control plant height with the purpose of increasing grain yield or biomass accumulation.

P193

Proteomic profiling suggests translational control is a key component of pollen tube germination in maize

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Germination of the pollen tube in maize occurs very rapidly in vitro, with the majority of pollen grains germinating after only 15 minutes on media. Transcriptomic analysis via microarray of mature and in vitro germinated pollen found no significant differences in transcript abundance between the two stages. Application of Actinomyocin D, which inhibits RNA synthesis, does not prevent pollen tube germination; however, treatment of pollen with Cycloheximide, a translational inhibitor, prevents germination. This suggests that *de novo* translation of peptides, rather than activation of new transcription, is a key facet in the control of germination. Comparison of proteomic profiles from mature and germinated maize pollen supports this hypothesis, and additionally suggest that protein degradation plays a role in pollen tube germination. To explore statistical methods for quantitative assessment of spectral count data from proteomic profiling, five different statistical packages in R have been used to analyze the counts for significant differences in peptide abundances between mature and germinated pollen. The results from these analyses will be compared to validation data obtained by western blotting, using selected antibodies to pollen proteins, to help define the package that is most accurate in modeling the biological response. We expect that a full and accurate analysis of the proteomic data should help identify key components acting in regulation and implementation of pollen tube germination.

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P194

QTL Analysis and Characterization of a Dominant Tassel seed Mutant, Ts*228

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Maize plants have separate male and female flowers: ears bear pistillate flowers and abort staminant organs while tassels bear staminant flowers and abort pistillate organs. Tassel seed (*ts*) mutants have feminized tassels with silks in the tassels indicating a failure of sex determination. Cloned tassel seed mutants include genes that encode a microRNA (*ts4*), a target of a microRNA (*Ts6*) (Chuck et al., 2007), a lipoxygenase (*ts1*) (Acosta et al., 2009) and a dehydrogenase (*ts2*) (Delong et al., 1993). Ts*228, a dominant tassel seed mutant, arose from EMS mutagenesis of A619. In a cross of Ts*228/B73 to the IBM recombinant inbred lines we were able to map several QTL with LOD scores of more than 3.5 for two traits: number of tassel branches (BN) and number of feminized tassel branches (FBN). We identified 6 QTL for BN and 3 for FBN. Ts*228 is highly expressive in B73 but suppressed in Mo17.

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P195

Quantifying maize *Sucrose transporter1* expression in different cell types from RNA in situ hybridizations

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One method to determine the cellular expression pattern of a gene is to perform RNA *in situ* hybridizations, with the RNA typically detected via precipitation of a colored product. However, the conclusions from these experiments are generally qualitative rather than quantitative. We have been studying the function of the maize *Sucrose transporter1 (Sut1)* gene, which functions to load sucrose into the phloem in leaves. Based on similarity to phloem loading SUTs in other plants, we hypothesized maize *Sut1* would be expressed in the phloem companion cells and/or sieve elements (CC/SE). To test this hypothesis, we performed RNA *in situ* hybridizations on mature leaf tissues. Maize *Sut1* RNA was indeed expressed in the CC. Surprisingly, we also detected the RNA in additional leaf cells that, in some instances, appeared to show stronger staining intensity. To characterize the magnitude of the expression differences between the CC versus these other cell types, we quantified the relative signal intensities between them using ImageJ. Here, we describe a method for using the "Color Pixel Counter" plugin to obtain quantitative signal expression data from microscopy images. In the large veins, the expression level in the CC (measured within the CC/SE area of the phloem) was ~67% relative to that of the other cell types. By contrast, the expression level in the other cell types was 63% and 38% relative to that of the CC in the intermediate and small veins, respectively. The progressive difference in the relative signal intensity across the vein classes appeared to be due to the increasing percentage of the area represented by the weakly-expressing bundle-sheath cells within the non-CC/SE cell types expressing *Sut1*. Our in situ expression data suggest maize Sut1 functions in both phloem loading of sucrose in the CC and in sucrose retrieval from the apoplasm in non-conductive cell types.

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P196

Regulation of cell fate acquisition by lateral organ patterning and boundary formation

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Our goal is to understand how cells fated to be lateral organs adopt specific cell types through patterning and subsequent cell type boundary differentiation. Genes regulating the blade-sheath boundary also function at other boundaries throughout the maize plant (Johnston et al., 2014). Previously, we generated an antibody against Liguleless1 (LG1) protein and characterized its accumulation at the blade-sheath boundary as well as tassel branch-rachis boundaries (Lewis et al., 2014). We created antibodies against the LG1 regulator, Wavy Auricle in Blade1 (WAB1), and against Liguleless2 (LG2), a positive regulator of tassel branch number and ligule differentiation. We monitored protein accumulation in wild type and mutant backgrounds finding overlapping and unique expression patterns in spikelets and spikelet pair meristems in the ear and tassel, at tassel branch boundaries and at blade-sheath boundaries. WAB1 and LG2 also overlap with Ramosa2 (RA2) accumulation in lateral domains of developing tassel branches and spikelets and spikelet pair meristems. LG2 and WAB1 overlap in spikelets and spikelet pair meristems in the ear as well. LG2 accumulation throughout the blade of WAB1 overexpressing plants demonstrates that WAB1 can positively regulate LG2. Our analysis suggests a conservation of gene regulatory circuits in many lateral organ boundaries throughout the maize plant. We propose experiments to uncover the intricate regulatory interactions between LG1, LG2, WAB1, RA2 and other factors to compare and contrast lateral organ patterning and tissue specification during the differentiation of leaves, ears and tassels.

Johnston R, Wang M, Sun Q, Sylvester AW, Hake S, Scanlon MJ. (2014). Transcriptomic Analyses Indicate that Maize Ligule Development Recapitulates Gene Expression Patterns that Occur during Lateral Organ Initiation. Plant Cell Dec 16.

Lewis MW, Bolduc N, Hake K, Htike Y, Hay A, Candela H and S Hake. (2014). Recruitment of regulatory interactions from the inflorescence to the leaf. Development, 141:4590-4597.

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P197

Study of the effects of the *br2* mutation on the shoot and the root system in the NC238 inbred line

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Plant height is an important agronomic trait: it can affect the ability of the plants to resist to lodging caused by wind, rain, or high plant densities and influence the addressing of energy to grain production instead of growing. Many dwarf phenotypes of crop species, including corn, wheat, rice and sorghum, have been described. In *Zea mays*, many brachytic mutants have been isolated but only one brachytic, called *br2*, was cloned and characterized. ZmBR2/PGP1/ABCB1 is the homolog of AtABCB1 auxin transporter protein. ABCBs, together with PINs, AUX1/LAXes, and PILSes act to generate an auxin polar transport (PAT) responsible of the establishment of an auxin concentration gradient in different tissues and/or at different development stages of the plant. ABCB1 functions in exporting auxin from intercalary meristems, and *br2* mesocotyls and coleoptiles exhibit reduced auxin transport (Knöller et al., 2010). *br2* plants are characterized by a shortening of the lower stalk internodes, however no remarkable alterations to the rest of the shoot are visible.

The NC238 inbred line, characterized by shortening of the lower internodes, has a *br2* mutation due to a novel transposon insertion in the ABCB1 gene. A tall revertant in which this transposon jumped away was isolated and used as a wild type reference for the NC238 inbred.

The root system of the two lines was compared measuring the root traits and testing the gravitropic response at seedling stage. Moreover, the root response to auxin transport inhibitors and auxin analog treatments was analyzed.

The mutant phenotype manifests at 7/8-leaves stage of the plant, and for better characterizing the mutant we performed expression analysis on the shoot tissues, in particular nodes and internodes, along with ABCB1 localization, expression analysis of PIN transporters, and auxin localization.

P198

Studying the effects of water deficit on maize inflorescence development

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For land plants, water availability is the most limiting factor for their growth and development. Our modern agriculture is also heavily dependent on water distribution. Drought has caused extensive loss to agricultural production worldwide every year and is expected to occur more frequently in important agricultural areas as a consequence of global climate change. A major component of drought that impacts plant growth is water deficit, however, how such stressful stimuli affect the developmental decisions of plants is poorly understood, especially in crop species. In crops like maize, most of the harvestable yield comes from gains derived from inflorescences, the tassel and the ear in the case of maize. Although the genetic processes that control the architecture of the maize ear and tassel are being studied, how drought affects these processes is still largely unknown. Here we analyzed the effect of early water deficit on the architecture and transcriptome of developing maize ear and tassel. Preliminary results show that ears enter a quiescence stage quickly after water deficit treatment, which is associated with a dramatic down regulation of the expression of many key regulators involved in inflorescence development. Despite these major changes in the transcriptional program of the ear, few developmental defects arise as a consequence of stress treatment and the transcriptional effects are reversed upon rewatering. The tassel inflorescence, on the other hand, shows distinct developmental changes from the ear. Tassels continue their growth during water deficit, although at a slower pace. Of particular interest, water deficit treated tassels form fewer basal branches, indicative of an architectural defect. These studies provide an important foundation for understanding the developmental mechanisms plants use to acclimate to stress and will be useful for identifying mutants and inbreds of maize that show phenotypic variation in this process for possible crop improvement.

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P199

Suppressor of sessile spikelet 3 functions in the production of paired spikelets

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The maize tassel has a main stem and long branches that are covered in pairs of short branches, called spikelets which are produced in pairs and bear the florets. Production of paired spikelets is a derived trait found in all 1000 species in the Andropogoneae tribe but are absent from more distantly related grasses including rice and wheat. The Suppressor of sessile spikelet mutants are semidominant mutants characterized by the production of single instead of paired spikelets leading to gaps between the rows on the ear and a sparse tassel. An understanding of the role of the Sos genes will shed light on the evolution of this novel inflorescence character.
Severe Sos3 mutants often have unbranched tassels or tassels with a few very short branches and ears with barren patches. Sos3 has been mapped to chromosome 1 between bins 1:05-1:06. To better identify the inflorescence development pathway that Sos3 functions in, genetic interaction with the ramosa1 and ramosa2 mutants, which have defect opposite to Sos3, was tested. The ra mutants are characterized by tassels with excess branches, giving them a Christmas tree-like appearance. ra1 ears often have prolific branching in place of normal kernels on the ear, while ra2 ears commonly have extremely disorganized rows. Interestingly, the sos3; ra double mutant results provide evidence that sos3 acts in the ra pathway. Future work will focus on fine mapping and further characterizing the Sos3 mutant phenotype.

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P200

Sympathy for the Ligule, a QTL that regulates the response of Liguleless narrow to the environment

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The ability of plants to respond to their environment is well documented, but a genetic understanding of this response is often lacking. We are using the dominant Liguleless narrow (Lgn) mutant to understand the interaction of genotype by environment at a mechanistic level. Heterozygous Liguleless narrow mutants have shorter, narrower leaves, a shorter plastochron, and reduced fertility in B73 but are near normal in Mo17. A QTL called Sympathy for the Ligule (Sol) was identified that is responsible for most of the Mo17 rescue (Buescher et al., 2014). Near isogenic lines that are entirely B73 except the region of Sol, which is Mo17, survive warm temperatures whereas plants that are only B73 die. Fine-mapping led to a narrow interval for Sol containing four possible genes. To identify the gene, we examined the response of Lgn in different inbreds. Lgn also dies at high temperatures in Ms71 but survives in NC350. Rescue in CLM322 does not seem to be linked. Comparison of sequences in the interval suggest that a gene of unknown function is likely to be Sol. Ms71 and B73 share a similar haplotype while Mo17 and NC350 share a distinct haplotype. To further nail the causative gene, we carried out an EMS revertant screen and have identified transposon knockout lines for the genes in the interval. In addition, a transcriptome analysis revealed other genes that may be affected by Lgn, providing clues as to the loss of viability for Lgn in B73 at high temperatures.
Buescher, E.M., Moon, J., Runkel, A., Hake, S., and Dilkes, B.P. (2014). Genes, Genomes, Genetics 4, 2297-2306.

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P201

TB1 and other TCP transcription factors are targets for phytoplasma effector protein SAP11

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Phytoplasmas are obligate intracellular bacterial parasites of plants that induce dramatic changes in plant development, including proliferation of stems (witch's brooms) and the reversion of flowers into leaf-like structures (phyllody). These bacterial parasites produce virulence proteins (effectors), including SAP11 and SAP54, which promote the degradation of plant TCP and MADS-box transcription factors, respectively, thus altering leaf and flower development [1-4].

Maize Bushy Stunt phytoplasma (MBSP) is an economically important maize pathogen in southern USA and Latin America, predominantly in Mexico and Brazil. Infected plants show disease symptoms characterized by foliar chlorosis followed by reddening, shortened internodes, ear proliferation and bushy stunt development. We identified a SAP11 homologue in MBSP that causes witch's broom development if overexpressed in *Arabidopsis thaliana*. We observed interaction with TEOSINTE BRANCHED 1 (TB1) a maize TCP transcription factor that regulates shoot branching. We are currently investigating if MBSP SAP11 induces TB1 degradation leading to the bushy stunt disease symptoms of MBSP infected maize plants.

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P202

The abnormal stomata phenotype of the *discordia3* maize mutant requires two independent mutations

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Correctly oriented cell divisions are critical for the development of plant structures, tissues, and organs. The preprophase band (PPB), a cortical ring of microtubules that forms prior to prophase, determines the placement of the future cell wall during plant cell division. During cytokinesis, the phragmoplast coordinates the formation of the partitioning cell plate and mediates its connection with the mother cell at the cortical division site delineated by the PPB. In the maize *discordia3 (dcd3)* mutant, new cell walls are incorrectly positioned during asymmetric cell divisions critical for stomata development in the leaf epidermis. PPBs in these asymmetrically dividing mutant cells are disorganized. *dcd3* is an unusual mutant that requires two independent mutations, *dcd3a* and *dcd3b*, to visualize the phenotype. Positional cloning of the *dcd3a* and *dcd3b* mutations narrowed their respective mapping intervals to syntenous regions of chromosome 3 and 8. We hypothesized that the *dcd3* phenotype is a result of independent mutations to a pair of paralogous genes in which one member is located in the mapping interval on chromosome 3 and the other is located in the chromosome 8 interval. An examination of the gene pairs that meet this requirement revealed several promising candidate genes. Sequencing identified likely deleterious mutations in the maize homologues of the microtubule severing protein, katanin p60. If loss of katanin activity is confirmed to be the cause of the *dcd3* phenotype, our analysis has identified new role for katanin in stomata formation in maize.

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P203

The B-class genes in maize: targets and timing in floral development

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The B-class MADS box genes are deeply-conserved regulators of second and third whorl (stamen) development in the flowering plants. This functional conservation does not help to explain the incredible morphological diversity observed in angiosperm flowers. The targets and interactors of the floral MADS box genes, including the B-class genes, present likely candidates in the search for the molecular underpinnings of diversity. We have been investigating the genome-wide roles of the maize B-class gene *sterile tassel silky ear1* (*sts1*) using RNA-Seq and ChIP-Seq. Our experiments have begun to reveal a complex transcriptional regulatory network centered around the B-class MADS box genes in maize.

P204

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 vegetative 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 the 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 has defects in tiller bud development and 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 required for AM formation and that these mutants provide an essential tool to dissect the gene regulatory network modulating AM production.

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P205

The dynamics in AN3 protein complex composition in growing maize leaves reveal how the balance between GROWTH-REGULATING FACTOR1 (GRF1) and GRF10 regulates the transition between cell division and cell expansion

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Most molecular processes during plant development function with a particular spatio-temporal specificity. So far it remained technically challenging to capture the dynamics in protein-protein interactions within a growing organ, where an interplay between cell division and cell expansion is instrumental. Here, we combined high resolution sampling in the leaf of Zea mays with tandem affinity purification followed by mass spectrometry (TAP/MS) of protein complexes. The growth controlling SWI/SNF chromatin remodeling complex associated to ANGUSTIFOLIA3 (AN3) was found to be strongly conserved within growing organs and between dicots and monocots. Moreover, we were able to demonstrate the dynamics in the AN3 interactome within the growing leaf as the co-purified GROWTH REGULATING FACTORs (GRFs) are organ- and even growth process-specific. Indeed, GRF1 was exclusively identified in samples enriched for dividing cells, while GRF10 was present in samples containing both dividing and expanding cells. These dynamics in protein complex composition were reflected at mRNA and protein level, showing a tight developmental regulation for the AN3 associated chromatin remodeling complex. In addition, the phenotypes of maize plants overexpressing a miRNA396a resistant GRF1 allowed for proposing a model how the association of the chromatin remodeling complex with specific GRFs tightly regulates the transition between cell division and cell expansion. Together, our data show how the technological advance to move from static to dynamic protein-protein interactions in a growing organ adds an additional layer to explain how important developmental switches are regulated.

P206

The maize *EXTRA GLUME1* gene regulates spikelet meristem development and microspore maturation

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Spikelet development is different in maize and rice as the monoecious maize plant forms separate male and female flowers upon maturation by the abortion of the pistil and stamen, respectively, while rice produces bisexual flowers. In rice, the *EXTRA GLUME1* (*EG1*) gene encodes a lipase which plays a key role in spikelet development.To test whether the function of the orthologous *ZmEG1* gene is conserved in maize, it was ectopically overexpressed in rice. The transgenic *Ubi1:ZmEG1* plants exhibited severe spikelet developmental defects, including alteration of the spikelet organ number, formation of ectopic floral organs in each organ whorl or in extra whorls and the appearance of indeterminate floral meristem. Additionally, the transgenic plants were male sterile due to defective tapetum cells and collapsed microspores. Shown by in situ hybridization, *ZmEG1* was expressed strongly in the inflorescence primordia, the tapetum cells, and microspores, but weakly in developing floral primordia, supporting its role in early spikelet development upon the expression *Ubi1:ZmEG1*. Noticeably, *ZmEG1* was also detected in degenerating stamen primordia but not in pistil primordia in the lower floret of ear spikelet, which may supply a clue about *ZmEG1*`s potential role in sex determination process. Taken together, our results suggest that *ZmEG1* is essential for spikelet development and pollen maturation.

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P207

The maize male sterile *fuzzy tassel* mutant makes abnormal stamens that fail to produce mature pollen.

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The maize *fuzzy tassel* (*fzt*) mutant is caused by a mutation in *dicer-like1* and has broad developmental defects. *dicer-like1* encodes a key enzyme for microRNA (miRNA) biogenesis and many miRNAs are reduced in *fzt* plants. *fzt* plants are shorter in stature than normal siblings and have shorter, narrower leaves. *fzt* also has striking inflorescence defects; inflorescence meristems are fasciated and other meristem types in the inflorescence are indeterminate. *fzt* is male and female sterile. *fzt* does not initiate obvious stamens in all inbred backgrounds, but in inbreds that do, the stamens develop abnormally. To understand the cause of male sterility in *fzt*, we compared stamen development in *fzt* and normal siblings. *fzt* stamens are smaller than normal siblings, often have twisted and shriveled locules, and range in color from yellow to dark brown. In contrast, normal sibling stamens have uniform smooth, yellow locules. To further investigate the *fzt* stamen defects, we compared development of *fzt* and normal siblings stamens in fixed, sectioned tissue. Early stamen development in *fzt* was indistinguishable from normal siblings. Later in development, however, *fzt* stamens had enlarged tapetum, shriveled pollen, and collapsed locules. Some *fzt* stamens contained pollen that appeared morphologically normal, but most of the pollen was not viable based on Alexander staining. Normal pollen is all tricellular at maturity; *fzt* pollen was a mixture of uni-, bi-, and tricellular pollen, indicating pollen development was arrested at multiple developmental stages. Pollen in normal siblings is loaded with starch before dehiscence. *fzt* pollen, however, failed to accumulate starch, suggesting that even pollen that developed to late stages arrested before maturity. We hypothesize that misexpression of specific miRNA targets underlies the *fzt* stamen defects and are currently examining expression of miRNA target genes with known roles in stamen development in other plants.

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P208

The maize YABBY transcription factor *drooping leaf1* (*drl1*) and its enhancer *drl2* regulate midrib and carpel development

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A major question in plant biology remains what genetic factors determine grass leaf architecture *e.g.*, leaf length, width, angle (blade deflection from the culm). Collectively, such morphological traits directly influence canopy structure and light penetration, photoassimilate production, and, important to crop systems, overall yield. We discovered and characterized a maize mutant with altered leaf architecture we named *drooping leaf* (*drl*), as leaf blades are midribless. Additionally, gynoecium development is severely compromised: unfused carpels encompass over-proliferative nucelli, akin to the pleiotropy observed for rice *dl* mutants. These mutant phenotypes are drastically enhanced by a modifier locus in Mo17. We cloned the underlying gene, *drl1*, and its paralogous enhancer, *drl2*, using positional cloning and generated a second *drl1* allele by *Ds* remobilization. The genes encode the maize *CRABS CLAW* ortholog, a putative transcriptional regulator with zinc-finger and YABBY domains. Sequence variation at the *drl2* locus in Mo17 likely enhances *drl1* mutant phenotypes; additional natural variants of *drl2* are currently under investigation. *In situ* hybridizations indicate *drl1* and *drl2* transcripts are absent from the central domain of the vegetative shoot apical meristem, but are detected in the incipient primordium, young leaf primordia and in reproductive organs. The apolar expression patterns of *drl1* and *drl2* in developing leaf primordia together with histological analyses suggest that these genes promote differentiation of a specific cell type, the clear cells, in the central midrib. Partial rescue of midrib and carpel phenotypes in *drl1-R; drl2-Mo17; Liguleless3-O* (*Lg3-O*) triple mutants reveals *Lg3-O* is likely epistatic to *drl1* and *drl2*. In floral tissues, *zea agamous1* interacts synergistically with *drl1; drl2*: triple mutants develop indeterminate branch-like structures in the axils of bracts, indicating these genes redundantly promote floral meristem determinacy. Our data suggest a conserved mechanism where DRL proteins regulate proper development of important agronomic traits in leaf and floral organs.

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P209

The SHORTROOT Signaling Pathway and Cellular Patterning in Monocots

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Approximately 15 different species of crop plants account for over 90% of the world's food calories, with just 3 species, rice, maize and wheat contributing 60%. The large-scale production of cereals has produced a steady increase in yields, however the world's population will soon outpace production. Increasing the yields of C_3 cereals through the introduction of C_4 photosynthesis is one potential pathway to increased plant productivity and water use efficiency. One of the keys to this transformation is an understanding of how vascular spacing is regulated and cell fate decisions are made in the development of mesophyll and bundle sheath cells. Recently the SHORT-ROOT transcription factor, originally describe in root development in *Arabidopsis thaliana* has emerged as a key regulator of Kranz anatomy. To determine how the SHR signaling pathway contributes to the cellular patterning of monocots, *Oryza sativa* (rice) and *Zea mays* (rice), we are currently examining leaf and root development in rice and maize lines with altered SHR signaling using the wealth of SHR data from Arabidopsis thaliana as a system for comparison.

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P210

The TANGLED lines of division

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Plants have two primary methods for establishing a body plan: division and expansion. Therefore, understanding cell division, particularly the coordination between cell division and differentiation mediated by correct orientation of the division plane, is crucial to understanding plant development. Although there has been recent progress in understanding the mechanical forces behind division plane orientation in plants, much less is known about the molecular factors regulating this process. TANGLED (TAN), a cortically localized protein with similarity to the microtubule binding domain of the tumor suppressor Adenoma polyposis coli (APC), promotes proper orientation of the division plane in plant cells. Intriguingly, APC promotes proper orientation of division planes in some animal cells and localizes to the cell cortex, similar to TAN. Live cell imaging was used to analyze the structure and dynamics of division structures in the maize *tangled* mutant identifying significant delays in disassembly of the cytokinetic apparatus and failure to return to the proper division site. Use of temporally regulated expression of TAN-YFP by ubiquitin-mediated proteolysis in maize *tangled* mutants demonstrates that TAN function is most important during early stages of the cell cycle. Together with the TAN interactors identified by yeast-two-hybrid and mass spectrometry, a temporally and spatially regulated division site interactome is proposed to mediate proper orientation of the division plane.

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P211

Transcriptional regulation of maize aleurone development by Nkd genes that code for ID domain transcription factors

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Cereal endosperm represents a major portion of human and animal caloric intake and has important industrial applications. The aleurone cells, that form the outermost layer of the endosperm, are the primary contributors of important dietary benefits of cereal bran and are also the major source of hydrolases paramount for the malting industry. The research described here explored the gene regulatory networks (GRN) controlling maize endosperm cell differentiation and development via transcriptional and molecular analysis of an aleurone development mutant, naked endosperm (nkd).The nkd mutation is controlled by duplicate genes of the Indeterminate (ID) domain protein family. The plant-specific ID domain is composed of four highly conserved tandem zinc fingers (zf); one standard C2H2 zf, one irregular C2H2 zf, and two C2HC zf's . Nkd mutant endosperm is characterized by defects in aleurone cell fate and differentiation traits as revealed by sporadic expression of aleurone identity markers, Vp1 promoter:GUS, and ABA resp.17-YFP transgenes. The DNA binding specificities of NKDs were revealed by SAAB and confirmed with GMSA. Results indicated that the NKDs' binding consensuses sequence (BCS) were similar to the previously characterized ID1 BCS in the recognition of a core TGTcGT motif. These results further indicate that NKDs have a slightly different BCS compared to ID1 and may indicate that the differences in primary amino acid sequence between NKDs and ID1 may represent important residues for conferring protein-DNA interactions. BiFC and Co-Pull down experiments demonstrate that NKDs homo and hetero-dimerize. A transcriptomic study involving the RNA-seq analysis of WT and nkd mutant was undertaken using endosperm cells captured by Laser Capture Microdissection (LCM). Pathway analysis revealed that NKDs are direct or indirect regulators of genes implicated in the control of gene expression, cell division/ differentiation, hormone signaling, carbon allocation and defense. A DNA motif enrichment analysis revealed that the differentially expressed genes in nkd mutant were enriched for NKDs BCS's and depleted for ID1 BCS in their promoter regions.

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P212

Transcriptome comparison of domesticated maize and teosinte reveals divergent coordination of the floral transition

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Optimization of the transition from vegetative to reproductive growth is critical for maximizing plant yield. Maize domestication and movement to higher latitudes required tropical varieties to shift from a short day photoperiodic induction of flowering to a day-neutral induction controlled mainly through autonomous signals. Teosinte (Zea mays spp. parviglumis), the wild ancestor of maize, will not flower under long day photoperiods, while temperate maize flowers regardless of photoperiod. The indeterminate1 (id1) gene is a key regulator of the autonomous pathway; loss of id1 function causes extremely late flowering in temperate maize, as well as floral aberrations. To better understand floral control in both teosinte and maize, Illumina RNA total sequencing was performed on mature (photosynthetic) and immature (non-photosynthetic) leaf tissue from florally induced and uninduced teosinte, and on mature and immature leaves from maize segregating the id1 mutation in the B73 inbred background. Induced teosinte mature leaf showed an increase in several Zea mays CENTRORADIALIS (ZCN) genes, which are signals related to FLOWERING LOCUS T and TERMINAL FLOWER1 in Arabidopsis. Consistent with the florigenic signal originating in mature leaves, most putative inductive signals were only detected in mature teosinte leaves. Curiously, the comparison between id1 and wild type mature leaves did not indicate comparable alterations in ZCN gene expression. Mutant id1 mature and immature leaves did however, show largely altered expression of carbon metabolism related genes, which mirrors previous observations. In the immature leaf there was evidence of altered plastid gene regulation, suggesting that ID1 may be involved the nuclear control of plastid function. This may reflect the shift from photoperiodic to day neutral flowering; temperate maize may be less reliant on ZCN based induction and in turn be more sensitive to autonomous carbon based signalling.

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P213

Uncovering the genetic toolkit underlying nitrogen nutrient foraging in Maize

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Efficient nutrient foraging in nitrogen poor soils is a critical, yet under-explored, aspect of nitrogen use efficiency (NUE) in crops. In this study, we exploit a split-root system to uncover molecular components controlling root nitrogen (N) foraging in a cross-species study of maize and Arabidopsis. In the split-root system, roots of a single plant are exposed to two distinct N-environments: N-replete vs. N-deplete. As a result, plants exhibit a stimulation of lateral root outgrowth on the N-replete side, when the other root half encounters a N-deplete environment. Our preliminary results showed that the maize B73 line is an efficient forager – it has enhanced lateral root growth on the N-replete side, compared to controls exposed to homogeneous N conditions. This N-foraging response we observe in maize is also conserved in Arabidopsis. Our previous transcriptomic study of Arabidopsis split root experiments, revealed that the root nitrogen foraging is mediated by a root-shoot-root signal relay, involving a cytokinin-dependent nitrate signaling. Our expansion of this genomic approach to maize lines that vary in NUE, will enable us to understand whether and how the underlying regulatory mechanisms of root foraging is conserved across species, and how it contributes to NUE. This unique split-root experimental setup will uncover mechanisms underlying two important contributors to NUE: i) a plants' ability to forage for nutrients in a heterogeneous soil environment and ii) shoot-root communication mechanisms of N-status. The identification of conserved genetic toolkits that are essential for root nitrogen foraging in maize and Arabidopsis will enhance translational studies of NUE in crops.

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P214

Understanding maize Gα signaling in shoot meristems, are additional receptor-like proteins involved?

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Shoot development proceeds by the successive initiation of new leaves, axillary meristems and flowers from pools of stem cells called meristems, which require subtle regulation and constant communication between cells. Stem cell regulation and communication is controlled by a core pathway, which involves a negative feedback loop between the CLAVATA pathway and the WUSCHEL homeobox gene. CLAVATA (CLV) signaling involves a secreted peptide, CLV3, and its perception by leucine-rich repeat (LRR) receptors, including the CLV1 leucine-rich repeat receptor like kinase (LRR-RLK), and an LRR receptor-like protein, CLV2.
Recently, we have shown that the maize COMPACT PLANT2 (CT2) gene, which encodes the predicted α subunit of a heterotrimeric GTP binding protein (Gα), functions in the CLAVATA pathway to control meristem size through its interaction with FASCIATED EAR 2 (FEA2), a homolog of CLV2 (Bommert et al, (2014), Nature, 502:555-558). Genetic data indicate that ct2 is epistatic to fea2, and co-immunoprecipitation and gel-filtration data further suggest that FEA2 and CT2 are in the same complex. Interestingly, FRET, BiFC and domain swap data experiments suggested that FEA2 and CT2 do not directly associate with each other, indicating other proteins may bridge their interaction. In order to find the bridging protein, we used IP-Mass Spec to find proteins that can be pulled down by both FEA2 and CT2. We identified an uncharacterized LRR-RLK that interacted with both FEA2 and CT2, and the results were confirmed by Co-IP experiments using the tobacco transient expression system. Furthermore, BiFC results suggested that the LRR-RLK directly associated with CT2, but the kinase domain truncated version of the LRR-RLK did not, suggesting the kinase domain is required for this interaction.
Our research facilitates the understanding of SAM regulation by introducing a new LRR-RLK in CLAVATA and G protein signaling. This LRR-RLK is preferentially expressed in meristems, and we are now searching for phenotypes, to understand its biological function in meristem development.

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P215

Using maize genes to improve the agronomic properties of orphan African grain crops

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Despite being grown for centuries and feeding millions of people each year, many orphan African grain crops are in desperate need of genetic improvement. These crops, including Tef and millet, are drought and flooding tolerant, display better water usage, and are nutritionally superior to maize. Despite this, they have several drawbacks, including low grain yields and modest forage quality. These problems often result from overproduction of tillers that drain valuable resources away from the main shoot, as well as increased lodging that causes seed loss.

Previous studies have identified a pair of microRNAs as important regulators of tillering. One of these, the maize miR156 gene *Corngrass1*, targets *SBP-BOX (SBP)* transcription factors and increases tillering when overexpressed. A different microRNA, miR393, targets auxin receptors and also increases tillering when overexpressed in rice. Expressing microRNA resistant versions of these target genes can have a major impact on the agronomic qualities of crop plants. For example, overexpressing miR156 resistant versions of *WEALTHY FARMERS PANICLE (WFP)* in rice increases seed yields by over 25% by reducing both tillering and lodging.

We are attempting to replicate the *WFP* phenotype in orphan grain crops by suppressing microRNAs involved in tillering and overexpressing miR156 target genes. We developed transformation protocols for cultivated Bridger tef (*Eragrostis tef*) and foxtail millet (*Setaria italica*) to test our constructs. Thus far, we have suppressed miR393 in *Setaria* and observed a reduction in tillering. In addition, we overexpressed the maize *unbranched3* miR156 target gene in Tef and observed decreased lodging as well as decreased aerial branching. We are currently introducing miR156 resistant versions of *unbranched3* into both species with the goal of increasing the severity of these phenotypes. With these genetic tools in hand we hope to help breeders establish these plants as viable alternative crops for the developing world.

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P216

Using natural variation and forward genetics to extend genetic networks controlling maize inflorescence development

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In recent years, several factors controlling maize inflorescence development were discovered, such as RAMOSA (RA) genes, which inhibit branching, and FASCIATED EAR (FEA) genes, which control meristem size and kernel row number. In order to better understand how these genes function and to uncover novel regulators, we used EMS mutagenesis to find enhancer mutations of the classical branching mutant ra3, which encodes a trehalose phosphate phosphatase (TPP), and leveraged the power of natural variation to identify modifiers of ra3 and fea2.

We mapped a number of EMS-induced ra3 enhancer mutations, and found that two independent lines contain mutations in the same enhancer, which we have cloned and will present, along with its possible mechanism of action. Other tentatively mapped mutations point to a role for RNA binding in RA3 signaling.

In a parallel approach, ra3 (in B73) and fea2 (in B73) were crossed to the NAM founder lines, and F2s were generated. For both mutants, we identified accessions that greatly enhance the severity of the mutant phenotype. A major fea2 enhancer locus from NC350 has been mapped using both BSA and screening of F2 populations made using the NAM RILs. Fine-mapping is currently underway, and will be presented. Similarly, the ra3 phenotype is enhanced in the Ki11 background, and we are also using BSA and NAM RIL F2s to map underlying loci.

Combining these approaches, we aim to extend our knowledge of the genetic mechanisms that control inflorescence development. Considering the importance of cereals for food and feed production, modulation of these genes holds great agronomic potential.

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P217

Engineering a Synthetic Centromere in Maize

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Increasing world population demands new technologies for crop improvement. Introducing a few transgenes into agricultural plants have helped combat pests and increase yields, but traits necessary to meet future demand will most likely require large sets of genes that cannot be easily introduced and manipulated by current methods. Artificial chromosomes are an emerging strategy to stack transgenes, but reliable centromeres are needed to faithfully segregate these constructs through cell division. We are bypassing the complications of epigenetically controlled natural centromeres, and engineering a synthetic centromere in maize that autonomously segregates an artificial chromosome. Synthetic centromeres have been created in other organisms by tethering a kinetochore protein to a specific DNA array via fusion with a DNA-binding domain. We are using a similar approach, fusing maize kinetochore proteins to DNA-binding domains and assaying their recruitment to an array inserted in the genome. The functionality of the synthetic centromere is measured by its ability to segregate an otherwise acentric chromosome fragment. Chromosome segregation is determined by imaging live meiosis and mitosis, and by the segregation of a pigment gene linked to the repeat array. If successful, synthetic centromeres could provide reliable segregation of platforms for transgenic traits that can improve plant health against diseases and changing weather patterns,and increase yield and resource efficiency.

Funding acknowledgement: National Science Foundation (NSF)

P218

Fast-Flowering Mini-Maize: Seed to Seed in 60 Days Update

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Two fast-flowering lines of maize were bred to have traits conducive to a short generation time. Fast-Flowering Mini-Maize (FFMM) can routinely be harvested at 60 days, easily producing five generations per year, though six generations may be possible under optimal conditions. Because of its short stature FFMM plants can be grown closer together than most lines and in smaller pots. FFMM was derived from hybrid of Neuffer's Early ACR by Alexander's Early Early Synthetic crossed to a selected F1 of Tom Thumb Popcorn by Gaspe Flint. FFMM A and B are derived from two independent plants and selected through 11 generations of selfing with selection for fast flowering, rapid seed maturity, high seed count, and good pollen yield. Being independently derived, a cross between FFMM A and B shows heterosis. Genetic markers y1 and R-scm2 have been introgressed into FFMMA to further its manipulation, and a FISH karyotype is available for both lines. FFMM A has also been Illumina sequenced and aligned to the B73 genome. FFMM can serve as a rapid cycling model system for research and teaching purposes.

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P219

Mutant alleles of a kinesin-14 class motor protein affect meiotic spindle formation in *Zea mays*

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Meiosis is the process of reductive cell division from which gametes are created. Microtubules form the spindle structure which is responsible for aligning chromosomes in metaphase and separating them in anaphase. The process of microtubule organization in meiosis is still not fully understood in plants which lack conserved microtubule organizing centers throughout their cell cycle. Zea mays (maize) has long served as a model species for plant cytogenetics and numerous spindle deficiency mutants have been identified in maize using genetic screens. One such mutant, DIVERGENT SPINDLE1 (*dv*), is characterized by unorganized spindles in male meiocytes which branch apart rather than focus at a point. Two different alleles of *dv* have been identified that share the divergent phenotype, *dv1* and *dv-IG*. Preliminary evidence links these two alleles to SNP mutations in maize Kinesin 6 (ZM-Kin6), a member of the kinesin-14 class of motor proteins. Presented are the results of a complementation test of the two alleles. All heteroallelic mutants (*dv1/dv-IG*) show spindle defects which match the homozygous mutant phenotype (n=7). Interestingly, some meiocytes heterozygous for the mutation (*dv1/+*) show spindle defects as well, indicating that this gene may have some sort of dominant phenotype. This is consistent with pollen viability data for these mutants generated previously. Future efforts will focus on further characterization of ZM-Kin6 to better understand the mechanism by which this motor works to focus spindle poles in these cells.

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P220

Production and use of colchicine derivatives to study mechanisms of plant meiosis

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Meiosis is a complex genetic process that results in haploid daughter cells required for fertilization. In plants, low dose treatments (0.1 mM) of colchicine are known to disrupt this process by interfering with chromosome interactions and crossovers during meiotic prophase. Colchicine is a secondary metabolite found in the corms of *Colchicum autumnale*, and is currently used both as a pharmaceutical treatment for gout and as an experimental tool to study cell division. Colchicine inhibits microtubule polymerization by binding to the α-β tubulin heterodimer. Whether the meiotic low-dose colchicine disruption acts through tubulin or another target unique to plant meiosis remains unknown. To explore this, we have synthesized two colchicine derivatives: Cy3-colchicine, a fluorescent variant for microscopic analysis, and biotin-colchicine, for biochemical purification of the cellular target. Preliminary studies using lily and maize meiotic cells show colchicine staining in the cytoplasm and at structures resembling the nuclear envelope (NE). This NE location is intriguing and is consistent with a model in which telomere-NE connections are required for chromosome interactions and proper progression of meiosis. These experiments could shed light on aspects of crossover control and mechanisms relating to the telomere bouquet and the nuclear envelope in meiotic chromosome segregation. Experiments are also underway to determine whether low-dose colchicine treatments disrupt crossovers in maize, as has been previously shown in lily.

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P221

Towards identifying SUN-interacting proteins in the maize nuclear envelope

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The nuclear envelope (NE) is a multi-functional structure that organizes the nuclear genome, facilitates nuclear migration, reassembles itself after each mitosis, and plays an active role in meiotic chromosome behavior. Several of these functions involve SUN-domain proteins and their interacting partners. SUN-domain proteins on the inner nuclear membrane (INM) interact with KASH-domain proteins on the outer nuclear membrane (ONM) to bridge the nucleoskeleton with the cytoskeleton. Although plant SUN proteins have been identified in several species, their biological functions have only recently begun to be investigated. To better understand the SUN-bouquet pathway in meiosis, we produced peptide antibodies to ZmSUN2, a C-terminal SUN domain protein of maize, and found that it stains a novel structure "the meiotic SUN belt" at prophase (Murphy et al 2014, doi:10.3389/fpls.2014.00314). The meiotic SUN belt retracts to a half-belt type arrangement that overlaps with the zygotene telomere cluster. We also demonstrate the genetic disruption of the meiotic SUN belt in meiosis-specific mutants, *desynaptic* (*dy1*), *asynaptic1* (*as1*), and *divergent spindle1* (*dv1*). To learn more about the functions and mechanisms of SUN protein complexes in plants, we are taking a biochemical co-IP approach to pull down SUN and SUN-interacting proteins using antibody against ZmSUN2. These studies are expected to further define SUN-KASH protein complexes in maize and shed light on the role of the NE in coordinating cytoplasmic forces with chromosomal dynamics.

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P222

Understanding how higher-order chromosome structure influences recombination in maize

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Meiotic recombination results in the exchange of DNA between homologous chromosomes, creating new allelic combinations that are transmitted through gametes to the next generation. In most organisms, cross-overs (COs) are not distributed evenly across the genome. Most maize COs are found in the distal ends of chromosomes, whereas the centromeric and pericentromeric regions are often devoid of COs. About 85% of the maize genome consists of repetitive DNA and a large portion of this DNA forms the constitutive heterochromatin that lies in proximal regions of chromosomes. It has been hypothesized that COs must be suppressed in these areas of highly repetitive elements to preserve genome stability. However double-strand breaks (DSBs), whose formation initiates the recombination pathway, do not follow the same pattern of CO distribution. Instead, maize DSB hotspots occur along the entire length of chromosomes, with no preference for distal or proximal regions. Our goal is to understand how chromosome structure differs between distal and proximal regions, and how these differences in structure relate to recombination patterns. We hypothesize that the constitutive heterochromatin is structured in such a way as to prevent CO formation in proximal regions. To test this hypothesis, we are examining the spatial and temporal localization patterns of RAD51, a protein critical in the repair of meiotic DSBs, along chromosomes. We are examining RAD51 occurrence in both repetitive and genic regions, and how these localization dynamics vary throughout the stages of early prophase I. Furthermore, we are investigating the behavior of RAD51 at various stages of chromosome restructuring. These data can provide evidence of different environments of higher-order chromosome structure between distal and proximal regions, which could allow homologous recombination to occur more readily in genic regions, and thereby suppress CO formation in repetitive regions.

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P223

Maize Genetic Resources in the Global System: The CGIAR Maize Germplasm Collections at CIMMYT (Mexico) and IITA (Nigeria)

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The Consultative Group on International Agricultural Research (CGIAR) Research Program for Managing and Sustaining Crop Collections supports 11 germplasm banks throughout the world, two of which, CIMMYT (International Maize and Wheat Improvement Center in Texcoco, Mexico) and IITA (International Institute of Tropical Agriculture in Ibadan, Nigeria) have maize collections. The Global Crop Diversity Trust, the organization that manages this funding, is supporting the development of a Maize Germplasm Conservation and Use Advisory Committee, which will meet for the first time at the 2015 Maize Genetics Conference. The Maize Global Conservation Strategy, drafted in 2006-2007, recognized the need for a 'small, forceful international committee to examine the needs for the entire maize germplasm community and be willing to fight to make it more effective and responsive.' We begin this work now, starting with the CGIAR-held maize collections at CIMMYT and IITA. The CIMMYT collection currently holds 24,067 landrace accessions, 3572 improved accessions, including the CMLs (inbred lines developed by CIMMYT breeders), 267 teosintes (Zea spp., excluding maize), and 161 Tripsacum genotypes. The CIMMYT online ordering system is at http://www.cimmyt.org/en/order-seed/maize-or-related-species-purpose/maize-germplasm-bank-menu IITA maintains a total of 1500 maize accessions from 20 different African countries with 30 % of the collection coming from the Republic of Benin and Nigeria. A total of 713 accessions have been duplicated for safety back up in the Global Seed Vault in Svalbard and 406 accessions in CIMMYT for secondary-level back up. Morphological characterization using the IBPGR Maize descriptors has been carried out on 700 accessions. The IITA maize database can be accessed online at http://genebank.iita.org/

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P224

Training Students in Analyzing "Big Data": a Case of Plant Stress Response

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Although many biology fields rely on analysis of large datasets, most undergraduate students consistently show lack of skills in analysis, graphical presentation, and interpretation of large datasets. To introduce "big data" into standard biology curriculum, we developed, implemented, and assessed a series of laboratory exercises on RNA-Seq data analysis that provides students with authentic research experiences. Maize is a thermophilic plant species that is highly sensitive to low temperature at all stages of development. Understanding how maize plants respond to cold and discovering genes responsible for cold-resistance is important for developing novel cold-resistant maize varieties. We created a large RNA-Seq dataset that includes several maize inbreds subjected to various environmental stresses, such as cold, heat, high salt, and UV exposure. This dataset can be interrogated to answer a variety of questions. We developed curriculum materials (worksheets and case studies) that guide students through RNA-Seq data analysis and prepare them to ask and answer their own questions using the RNA-Seq dataset, elucidating stress response in mazie. Follow-up implementations and extensions of this project will be discussed, as well as the data on assessment of student learning and their evaluation of research experiences provided by this project.

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P225

A maize domestication QTL for ear internode length maps to a gene encoding for YABBY transcription factor.

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A TCP transcription factor, *teosinte branched1* (*tb1*), was previously identified as a maize domestication QTL of large effect contributing to the differences in plant and inflorescence architecture between maize and its progenitor, teosinte. Recently, it has been shown that there are several additional QTL tightly linked to *tb1* that also affect inflorescence traits under selection during domestication. One of these additional QTL is called *enhancer of tb1.2* (*etb1.2*), which interacts with *tb1* in regulation of ear internode length. We fine-mapped *etb1.2* to a ~68 kb region on the long arm of chromosome 1. This 68 kb region contains exon 1 of *ZmYAB2.1*, a gene encoding for YABBY transcription factor, as well as ~67.8 kb of 5' upstream sequence that does not include any annotated genes. Since there is no difference in amino acid sequences between maize and teosinte allele of *ZmYAB2.1* exon 1, a cis regulatory change is likely the causal polymorphism. This hypothesis is supported by evidence of selection for sequences around exon 1 of *ZmYAB2.1*. Further characterization of *ZmYAB2.1* involves quantitative PCR (qPCR) on maize and teosinte alleles of *ZmYAB2.1* and *tb1* in maize background, which shows no obvious interaction between *ZmYAB2.1* and *tb1*. However, the qPCR results suggest a positive correlation between *ZmYAB2.1* expression and ear internode length and a negative correlation between *tb1* expression and ear internode length.

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P226

A multi-institution collaboration to study the genotype-by-environment interaction in maize across a diverse set of hybrids and locations

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A multi-institution collaboration was established to study genotype-by-environment interactions in maize across a diverse set of hybrids and locations. A pilot study conducted in 2014 consisted of nearly 900 hybrids and 25 U.S. locations and one Canadian location. The experiment was highly unbalanced where many hybrids were only tested on a subset of locations, but 10 hybrids were common to all locations. Over 200,000 SNPs were scored on parental inbreds using genotyping-by-sequencing and synthetic genotypes of the hybrids were constructed through the genotypes of the parental inbreds. Data on seven environmental variables (temperature, precipitation, radiation, etc.) recorded every 15 minutes at each location were available. The objective of the current study is to evaluate the potential of predicting performance between and within environments through sharing information between hybrids and between environments. Two traits were analyzed: plant height and days to anthesis. To assess the advantages of including information of other hybrids tested in other locations, three cross-validation schemes (CV) were designed: (i) Leave-one-out CV (within trials), (ii) Prediction of relative hybrid performance in entirely new environments (CV0), (iii) Prediction of new hybrids in environments for data is available (CV1). Three genomic prediction models were fit: (1) E+G, (2) E+G+GE, (3) E+G+GE+GW, where E represents the environmental effect; G the genotype effect; GE the genotype-by-environment interaction effect; and GW the genotype-by-environmental covariate interaction effect. Results suggest that large phenotypic and genotypic datasets provide ample information for good predictions of new environments (CV0 and model 1). However, when information on the environment in question is available in the form of data from other hybrids (CV1), the accuracy was improved by up to 38%. Best results were obtained under the CV1 scheme when considering the interaction components (GE and/or GW), increasing the prediction accuracy up to 75% in some cases compared to CV0. These results highlight the importance of capturing GxE effects for predicting phenotype from genotype.

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P227

A parallel selection experiment aimed at studying the genomic response to geographical selection for flowering time in maize.

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Genetic diversity is a key component to the health and success of many major agricultural crops as it underlies the capacity for a crop to be bred for new conditions, but many crop production centers, such as the US Corn Belt, leverage only a narrow sample of diversity. Developing a deepened understanding of response to geographical selection could aid in capitalizing on new sources of diversity to address challenges of sustainable crop production. We conducted a parallel selection experiment: a single founder seed population derived from the systematic inter-mating of seven tropical inbred lines was phenotypically selected for early flowering time at eight separate locations spanning the near extreme latitudes of the USA. A structured sampling method was used to test for both allele-phenotype association and allele frequency response across generations. An imputation-less genotyping-by-sequencing (GBS) approach was developed and validated for genotyping heterozygous populations with complex or unknown parentage. A core set of ~15,000 SNP loci were identified that do no exhibit evidence of ascertainment bias, show expected patterns of segregation in F2 populations, and have >99.7% genotyping accuracy. We will present the latest results of this effort, including imputation-less GBS, genetic diversity and linkage disequilibrium features of the parents and base population used for selection, analysis of phenotype data on all of the selected populations evaluated at all of the selected locations, and preliminary genomic analysis of the parallel selection resource.

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P228

A tropical genome with a temperate phenome: inference on the genetic architecture of tropical-to-temperate maize adaptation

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To investigate the genomic basis of tropical-to-temperate adaptation, we applied a novel genetic design to dissect genetic architecture of response to selection in maize. Tests for footprints of selection and genotype-phenotype correlation on 50,117 SNP loci were used to study Hallauer's Tusón, a tropical landrace adapted to the temperate environment of Iowa by a decade of phenotypic recurrent selection for early flowering time. Previous research indicates that flowering time in maize is a quantitative trait under polygenic control with mostly small and some larger effect QTL/genes. Under the pressure of artificial selection, hundreds of SNPs across the genome exhibited patterns consistent with directional selection. Variation at and surrounding candidate genes for flowering time exhibited dual evidence of selection and phenotypic association. This study shows that selection at many loci throughout the genome, each with small effects on the trait, can produce large phenotypic changes in relatively few generations of artificial selection. Furthermore, in terms of increasing the genetic diversity of US corn, our study revealed that all ten generations of Hallauer's Tusón clustered with tropical rather than temperate germplasm, based on genotypic information, highlighting the potential for establishing new sources of temperate adapted germplasm.

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P229

Assessing the genetic diversity of public and private popcorn breeding programs

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As the market for popcorn continues to grow both in the US and internationally, it becomes increasingly more important to have access to breeding germplasm information. The intent of this project is to create a diversity panel consisting of popcorn lines from both public and private sources in order to better understand the population structure of the current US popcorn germplasm and to identify key genetic markers related to target popcorn alleles.
350 popcorn lines originating from both the USDA and from a private company will sampled and undergo GBS. These sequences will be analysed using the TASSEL-GBS pipeline and will be used to create a SNP panel geared toward popcorn varieties. This SNP panel will then be used to characterize the popcorn germplasms from both sources in order to identify SNPs associated with key alleles and/or individual genetic markers that will aid in future breeding programs. Markers discovered from the pipeline will also be used to characterize both germplasms in the context of the known United States maize population as a whole.

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P230

Beyond additive effects: identifying pleiotropic and epistatic factors contributing to carotenoid and tocochromanol metabolic pathway dynamics in the US-NAM panel

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The identification and characterization of loci associated with variation in carotenoid and tocochromanol metabolites provides molecular tools with enormous potential to substantially increase the availability of provitamin A and vitamin E in plant-derived foods. To date, our joint linkage-genome wide association study of carotenoid and tocochromanol grain levels in the US nested association mapping (US-NAM) population has found the majority of identified QTL to have gene ontologies related to carotenoid and tocochromanol synthesis and degradation, including prenyl group synthesis and amino acid biosynthesis. Previously described results from RNA-seq analysis from our laboratories suggest that many of these genes regulate metabolite levels through differential expression. Given that the measured traits originate from common metabolic precursor pathways, it was presumed that the major effect loci identified in our analysis would affect more than one trait, yielding pleiotropic effects. Sharedness of QTL effects across the measured traits was observed to be much higher for carotenoid traits than tocochromanol traits, indicating that carotenoid nutritional profiles are more responsive to pathway-wide changes than tocochromanol profiles. Unlike other traits studied in the US-NAM population to date such as flowering time, plant height, and inflorescence architecture, many significant pairwise epistatic interactions were detected among both carotenoid and tocochromanol QTL, supporting the phenomenon of biochemical epistasis, where the effect of one locus can be conditioned on the effect of another. Moreover, the hierarchy of epistatic interactions observed across metabolites suggests major regulatory points within the pathways. The intersection of pleiotropy, epistasis and expression QTL analyses for both carotenoid and tocochromanol traits points to similar modes of regulation across the pathways, albeit with different magnitudes of effects. Incorporation of additive, epistatic and pleiotropic QTL effects into marker based biofortification programs will greatly assist in achieving expected nutritional profiles.

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P231

Candidate gene discovery by analysis of natural variation for cell wall compositional traits in maize

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Maize stover is a near-term source of biofeedstock for biofuel production, and maize is an important model for other biofeedstock grass species. Our project exploits naturally occurring phenotypic variation for non-grain biomass traits in maize coupled with extensive genetic information to identify novel genes that can reduce biomass recalcitrance. We have evaluated cell wall bound glucose and pentose release from cores taken from the second lowermost stalk internode of 563 diverse maize inbred lines using an automated digestibility platform. These inbred lines were evaluated in 2010, 2011 and 2012 in field experiments with a randomized complete block design and two replications. Three representative plants per genotype and field replication were sampled each year. We observed significant genetic variation for both traits with relative amounts of sugar per mg dry biomass that range from 4.1 to 20.2 for glucose and 1.2 to 9.02 for pentose release across genotypes. The genetic basis of these traits was analyzed using a set of 438,222 SNPs in a genome wide association study (GWAS). We identified natural alleles of different genes that are promising candidates to alter biomass digestibility. Based on their biological relevance and expression profiles, genes were prioritized for validation. These candidates are annotated as playing a role in xylan biosynthesis and nuclear processes that relate to cell wall biosynthesis. Transgenic plants and transposon alleles will be used to further explore the potential of these genes in reducing biomass recalcitrance.

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P232

Characterization of QTL Influencing Seedling Cold Tolerance

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There have been several studies on cold tolerance for germination and early growth in maize. Cold tolerance in established seedlings has not been characterized as well. B73 and Mo17 seedlings grown in standard conditions for 14 days and then exposed to a 4C cold treatment show varying sensitivity to the cold. QTL mapping using the IBM RIL population identify two major QTL contributing to the differences in cold sensitivity. We used B73-Mo17 near-isogenic lines (NILs) to validate a major QTL located on chromosome 5. Several NILs containing introgressions of the cold tolerance locus were used to generate a population that would include recombinant individuals. This population was screened to identify novel crossovers that break up the introgression and narrow the region of interest and plants containing informative recombinant events were self-pollinated. The cold tolerance for the off-spring that segregate for the novel crossover events will be evaluated in order to fine-map the genetic basis of cold tolerance. In addition, expression profiling data will be used to further understand the potential role of gene expression differences for genes within the QTL region. This project will provide further understanding of the genetic basis for cold tolerance during vegetative growth.

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P233

Cloning and characterization of a multiple disease resistance QTL for both southern leaf blight and grey leaf spot in maize

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Southern leaf blight (SLB, causal agent *Cochliobolus heterosrophus*) and grey leaf spot (GLS, causal agent *Cercospora zeae-maydis*) are two major foliar diseases in many maize growing regions. Characterization of multiple disease resistance (MDR) QTL would accelerate the development of durable resistant cultivars using marker assisted selection. A previous study has identified a major MDR QTL on maize chromosome bin 9.02/03 using near isogenic lines (NILs) carrying introgressions from the maize line NC250 in the background of the standard maize line B73. A major resistance QTL for GLS was also localized in bin 9.02/03 in NIL populations carrying introgressions from maize's wild progenitor species teosinte in a B73 genetic background. A strong SLB QTL in bin 9.02 was also identified from the maize nested association mapping (NAM) population using 7386-marker linkage map. To fine map this QTL, NIL-derived mapping populations (donor region comes from NC292 or teosinte) were used to delimit QTLs for both SLB and GLS into the same small interval (<300 kb based on B73 genome). Genome-wide association study for SLB in NAM identified a significant SNP (RMIP=0.59) at 16,317,865-bp (AGP_V2) on chromosome 9 by using 28.5M combined SNPs and read depth variants from maize HapMap1 and 2. The significant SNP locates in the fine mapping region. The closet candidate gene underlying the GWAS hit encodes a caffeoyl-CoA O-methytransferase (CCoAOMT) that has been reported to participate in lignin biosynthesis in plants. Two separate mutants with an insertion in 3'-UTR region in the *ZmCCoAOMT* gene significantly enhanced disease resistance for both SLB and GLS in the field by increasing its expression. Transgenic validation of the *ZmCCoAOMT* is underway. A better understanding of molecular basis of MDR QTL might provide new insights into plant-pathogen-interaction and facilitate efficient deployment of the resistant alleles in different breeding programs.

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P234

Combined Mapping of Height and Flowering Time in Across 15 Biparental Populations using Both Traditional and Bayesian Association Mapping

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It is increasingly recognized that combining different datasets can provide much greater power for genetic mapping than any individual dataset can provide on its own. However, it is not always obvious how to combine disparate datasets, especially when samples are not shared across them. We jointly analyzed 15 biparental maize families from CIMMYT's Water-Efficient Maize for Africa (WEMA) project to identify quantitative trait loci (QTL) controlling height and flowering time under both well-watered and drought conditions. Importantly, these families were never designed to be analyzed together, and no single environment ever contained all of them at the same time. We take advantage of the common design to combine phenotype and genotype data across the families for traditional genome-wide association, and we also perform a Bayesian association analysis that does not rely on common factors but instead builds up from individual families in single fields. The results of these parallel analyses largely agree, and our Bayesian analysis provides a framework for combining data across even more disparate datasets. We also identify both known and novel QTL controlling these traits, and these methods will soon be extended to identify loci for water-use efficiency, drought tolerance, and and other targeted phenotypes in this population.

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P235

Comparative Phenomics in Plants

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Plant phenotype comparisons enable us to explore the relationship between gene function, sequence similarity and consequently to make predictions for crop improvements. Plant phenotypes are described primarily using free text and are inconsistent between species, making computer facilitated queries difficult; however, assessing this data manually is impossible due to the ever increasing plethora of available phenotype data. To address this issue, we undertook a pilot project to formalize phenotype descriptors for six plant species, focusing on mutant phenotypes associated with sequenced genes. To do this, we selected free text descriptions of 1742 unique phenotypes for 2741 genes from maize, rice, Arabidopsis, soybean, tomato, and Medicago and manually converted them into a common Entity-Quality format using taxonomically broad ontologies; e.g., the Plant Ontology and the Gene Ontology. The power of this method lies in the fact that hierarchical ontologies allow even distantly related phenotypes to be identified computationally. Similarity scores were calculated for each pair of phenotypes and the resulting associations were loaded into a database and query tool called PhenomeNet (http://phenomebrowser.net/plant/query.php). PhenomeNet enables researchers to query these associations with locus names, gene identifiers, and phenotypic descriptions. To the user, this is roughly similar to using BLAST to identify similar sequences. Although the phenotype dataset must be greatly expanded to be a useful research tool, our work demonstrates that comparing phenotypes among divergent plant species can be automated.

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P236

Competence Center for Doubled Haploid Research

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DH technology allows production of fully inbred lines in two generations as opposed to the six or more generations by conventional inbreeding. Other advantages include: reduced expenses to produce cultivars, more precise evaluation of phenotypic traits, effective elimination of undesirable genes, trait fixing in haploids using marker assisted selection leading to an effective use of molecular markers, and more efficient combination of transgenic traits. Because competitiveness of breeding programs depends on genetic gain per unit time, use of DH technology has become routine in breeding of the major cereal self-pollinated species, e.g., wheat and barley, and recently has become established in private and public (Doubled Haploid Facility: http://www.plantbreeding.iastate.edu/DHF/DHF.htm) sector breeding of maize and canola. However, in several major crop species including soybean and sorghum, protocols for development of DH lines are not available or are inefficient. Moreover, there is substantial potential for improving the DH production process in crop species like maize. In consequence, there is a need to better understand the molecular and cellular processes involved in haploid plant formation as well as genome doubling and their consequences, including reasons for genetic variation within and between species (Thrust 1). There is a need to establish DH technology, where it is not available currently (such as soybean and sorghum) (Thrust 2), and to make the different steps in DH formation more efficient including automated procedures (Thrust 3). Moreover, there is a need to develop novel strategies based on haploids and or DHs with regard to breeding of various crop species, pre-breeding, management of genetic resources, as well as development of novel types of experimental populations (Thrust 4). We will report on joint efforts of Iowa State University and University of Illinois Urbana-Champaign to establish a NSF I/UCRC Competence Center for Doubled Haploids Research (CeDHR: http://cbec.gdcb.iastate.edu/cedhr/).

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P237

Construction and use of NILAS resources to investigate barriers to maize improvement

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Tropical maize possesses favorable alleles for numerous traits that are absent from US maize but will be important for adapting to environmental challenges. The latitudinal cline in day length and temperature extending from Central America—the center of maize diversity—to the Northern US represents the most significant barrier to the introduction of tropical genetic diversity for corn improvement. Our goal is to deepen our understanding of the genetic factors preventing access and use of novel genetic variability harbored by tropical germplasm. Here we report our use of marker assisted selection to develop a Nearly Isogenic Line Allelic Series (NILAS) at each of four major photoperiod-responsive loci in the maize genome. Each NILAS comprises a large set of inbred lines harboring introgression segments at a single specific genomic locus across a range of functional alleles. In this case, we have incorporated alleles from seven tropical donor lines into both stiff stalk and non-stiff stalk temperate genetic backgrounds such that locus-specific introgression libraries are made for each of the 14 tropical X temperate contrasts. We discuss the technical details of this marker-assisted breeding effort as well as the range of questions that can be addressed using a NILAS.

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P238

Crowding stress genomics in sweet corn

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Crowding stress tolerance mechanisms in sweet corn are poorly understood. Our objectives were to 1) explore gene expression patterns among hybrids grown under crowding stress, and 2) identify linkages between phenotypic responses and gene expression patterns. Grown under conditions of elevated plant population density, three high-yielding hybrids and three low-yielding hybrids were grouped for transcriptional and phenotypic analyses. Transcriptional analyses identified from 426 to 937 differentially expressed genes (DEGs) for each hybrid. Large gene expression pattern variation among hybrids and only 31 common DEGs across all hybrid comparisons suggests each hybrid has unique responses to crowding stress. Biological functions of DEGs were similar among high-yielding hybrids, with large percentages of up- and down-regulated DEGs in enzyme families and proteins. Biological functions of DEGs varied more among low-yielding hybrids. Seven clusters of genes (modules) were identified between groups. Correlation and cluster analyses of modules and phenotypic response showed strong positive association between 1) yield traits and modules with up-regulation in high-yielding hybrids, and 2) plant traits and modules with down-regulation in high-yielding hybrids. Functional analysis of modules and common DEGs identified candidate mechanisms, including transcription regulation, DNA structure, cell wall degradation, hormone synthesis, glycolysis, and polyamine-involved primary metabolic pathways. Collectively, these mechanisms may be useful for improving sweet corn yield potential.

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P239

Diallel Cross Analysis for Methionine in Maize

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High methionine grain is useful for animal feed because most maize lacks sufficient methionine levels to meet nutritional demands. Methionine is a limiting amino acid in poultry diets so methionine supplementation is typically required and very costly. Several maize varieties with increased methionine levels have been developed which utilize the *dzr1* locus that results in elevated levels of the methionine-rich 10 kDa zein, the *floury-2* allele that encodes an unusual form of a major alpha zein, or recurrent selection to elevate total methionine content. To further our understanding of methionine regulation at the genetic level, a complete 7 x 7 diallel cross study including inbred lines with different approaches to increasing methionine content was carried out. We analyzed methionine content in the resulting 7 inbreds and 42 hybrids over a period of two planting seasons. Griffing's experimental method 3, model 1 was used to calculate GCA, SCA and reciprocal effects. Two general and four specific combining abilities were significant along with two reciprocal effects. In addition to the diallel study, a yield trial was conducted using hybrid combinations. A negative correlation between methionine content and yield was found. Although our results suggest mechanisms used to elevate methionine levels do not act synergistically in hybrid combination, breeders should consider each of the methods for increasing methionine as well as their interactions when breeding high methionine corn.

Funding acknowledgement: United States Department of Agriculture (USDA)

P240

Discovery of Maize Fe and Zn Environment-Homeostasis Associated QTL

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Iron (Fe) and zinc (Zn) are essential for plant, animal and human nutrition. Low Fe and Zn in crops and livestock results in their reduced health, which when consumed over prolonged periods, can in turn negatively impact the nutrition of human populations. Maize is grown worldwide, as a staple crop for some and a valuable commodity for others and has the potential to be a useful tool for targeting the dietary Fe and Zn deficiency among the undernourished poor. Maize's remarkable global spread is largely due to the degree of genetic and phenotypic diversity that can be harnessed into adaptation to local conditions. This study was performed on the Goodman Diversity Panel (GDP) with corresponding analysis of the nested association mapping (NAM) population, to take advantage of greater statistical power and resolution and to perform joint linkage (JL) and genome-wide association (GWAS) analyses of quantitative genetic loci (QTL) across 3 temperate and 2 tropical locations. Previous studies that have used a candidate-gene knockout approach have yielded narrower successes in identifying genetic determinants of ionomic homeostasis, whereas the NAM JL-GWAS approach, in this study, has borne significant QTL identification. Currently 227 JL ionomic-QTL have been identified, with successive rounds and accompanying GWAS data we anticipate discovery of good QTL for Fe and Zn homeostasis. A web interface for browsing the maize co-expression network has been developed for querying individual genes or large regions of the genome.

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

P241

Dissceting the genetic architecture of maize rind penetrometer resistance by joint-linkage and genome-wide association mapping

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Rind penetrometer resistance (RPR) is an effective way to measure stalk strength in maize (*Zea mays L.*). Selection for RPR causes divergent changes in several stalk traits and stalk lodging resistance in maize. In this study, we measured RPR across 1,887 lines of 10 independent RIL (recombination inbred line) populations (maize Random Open-parents Association Mapping population, ROAM) in four environments. With the three methods of separate linkage mapping (SLM), joint-linkage mapping (JLM) and genome-wide association mapping (GWAS), 31, 80 and 112 loci were significantly associated with RPR in ROAM, respectively. In addition, GWAS was performed to detect the associations for natural variation of RPR in an association panel consisted of 508 diverse inbred lines. A total of 81 SNPs in 22 loci significantly associated with RPR were detected at $P<0.0001$. Among these loci, 122 consistent loci for RPR were detected by at least two methods. These results indicate numerous minor-effect loci contribute to the complex nature of maize RPR.

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P242

Ecogeographically structured allele frequency analysis of maize landraces: examining the role of photoperiod sensitivity loci in the post-domestication spread of maize in the Americas

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Maize is cultivated across a diverse array of geographies and environmental conditions. Modification of photoperiod sensitivity was crucial to the post-domestication spread of maize across a wide range of latitudes, because sensitivity needs to be reduced to grow maize at higher and lower latitudes where day lengths are long during the growing season. While some genes important to photoperiod sensitivity are known, it is unknown which alleles are important for which environments. We have curated a panel of 372 accessions from 307 landraces collected throughout the Americas. For each accession, five plants were sampled for a total sample size of 1,860 plants, as well as 60 plants from 12 accessions of wild relatives. We are presently conducting a genotyping effort to test hypotheses about the role of specific flowering time genes and implicated nucleotide variants in the post-domestication spread of maize throughout the Americas. Genome-wide diversity is being assessed by an imputation-less genotyping-by-sequencing method that enables accurate scoring of heterozygous genotypes with unknown parentage. Sequence diversity across this random set of genome-wide markers is being compared to that at 17 key loci associated with photoperiod sensitivity. Candidate loci were selected based on compelling evidence that has emerged across previous studies on the genetics of photoperiod sensitivity. These genes and their upstream promoter regions, comprising ~72 Kb, are being sequenced using Ion Ampliseq, a method for preparing highly multiplexed sample and amplicon-specific PCR libraries. Using the resequencing data, we will examine the relationship between allelic diversity and geographical, as well as environmental factors.

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

P243

Effects Of A Single Historically Important Sweet Maize Inbred Used Ubiquitously In Breeding On Modern Elite Inbreds

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In breeding, crosses are made in order to shuffle the genome and then select for optimal configurations. During this process we occasionally find individual lines that are extraordinary. These individuals are rare, but have lasting effects in the germplasm. One well-known example of this is B73, which is present in the pedigrees of a high proportion of modern stiff stalk inbreds. In sweet maize breeding, a similar situation has occurred with the inbred IL677a. We believe this individual inbred is present in the pedigree of 100% of the known *sugary enhancer1* (*se1*) inbreds in modern sweet corn. A single locus was originally associated with IL677a, which has recently been fine mapped. With this, IL677a's parents, sister lines and a sample of derived inbreds were screened for the fine mapped *se1* allele, and IL677a was confirmed as the only original source of *se1* in the known germplasm. However, we now know that the original trait associated with IL677a is polygenic in nature. GBS and haplotype mapping of a sampling of modern elite sweet corn inbreds available at the University of Wisconsin-Madison sweet corn breeding program, has allowed us to look at the 1. the haplotype of the *se1* locus originally selected for in the background of all *se1* type sweet corn inbreds and 2. the whole genome size and frequency of IL677a haplotype blocks. Given the single major gene and polygenic inheritance from this historical parent, a high proportion of the genome, in large haplotypes, are preserved within the genome of modern elite lines. Comparisons to field corn and the use of B73 in much of the stiff stalk germplasm background will also be made.

Funding acknowledgement: USDA Hatch, University of Wisconsin-Madison

P244

Effects of elevated ozone on foliar and ear disease in maize inbreds.

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Predicted climate change, including the rise in tropospheric ozone (O3) in agricultural zones, poses a challenge for future global food supply. Free Air Enrichment Facilities (FACE) provide a tool for studying the effects of changing atmospheric conditions on crop performance in the field. Interactions between ozone and other abiotic and biotic stresses are not well understood. In particular, the ability of ozone to mimic the pathogen-induced oxidative burst can have different effects in specific pathogen-host systems.

A diverse panel of maize inbred lines, including the founders of the nested association mapping (NAM) population, were grown under ambient (40 ppb) and elevated (100 ppb) ozone concentrations at the SoyFACE facility at Champaign, IL, in 2014 and evaluated for naturally occurring fungal disease incidence. The most commonly observed diseases were Common rust (*Puccinia sorghi*; biotroph), Northern leaf blight (*Exserohilum turcicum*; hemibiotroph), and Brown spot (*Physoderma maydis*; biotroph). Foliar disease was evaluated on a 9 point severity scale at two time points, after fifty and eighty percent of the lines had reached anthesis. Fungal ear rots were evaluated on a 6 point severity scale directly following a staggered harvest scheduled 8-10 weeks after anthesis. Ozone effects on foliar disease incidence were modest and variable between pathogens and inbreds, with a trend towards lower Brown spot infection severity under elevated ozone. Effects of elevated ozone on ear rot were also strongly genotype-dependent, again with a trend towards lower ear rot severity under elevated ozone. This suggests that ozone exposure may lead to pathosystem-specific priming of defense responses.

Funding acknowledgement: National Science Foundation (NSF)

P245

Effects of elevated ozone on tassel and ear traits in diverse inbred and hybrid maize

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Tropospheric ozone is an important air pollutant. Ambient concentrations of ozone in 2000 were estimated to cause $US 11-18 billion in yield losses globally. And, concentrations of ozone are predicted to increase 20% by 2050, causing additional crop damage. Plant exposure to elevated ozone can affect metabolic pathways, speed up senescence, and damage reproductive structures all of which reduce crop yield. Despite the importance of C_4 crops such as maize, very little is known about their response to the oxidative stress caused by ozone. This study evaluated the effect of elevated ozone on tassel and ear traits. A diverse panel of maize inbred and hybrid genotypes, including founder lines of the nested association mapping population, were fumigated at the SoyFACE facility with 100 ppb ozone using Free Air Concentration Enrichment (FACE) technology. A 5-point scale was used to score the extent of tassel skeletonization. Images of ears were captured and analyzed as a high throughput method of evaluating traits including ear length and width. Elevated ozone caused significant decreases in ear length. There was significant genetic variation in response among both inbred and hybrid genotypes. The role of changes in kernel row number and individual kernel size will be presented.

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

P246

Effects of ozone on maize ear architecture

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Tropospheric ozone (O3) is estimated to cause billions of dollars in global crop losses, but few studies have investigated the effects of elevated ozone on growth and development of C4 crop plants. The goal of this study was to understand how maize ear characteristics are affected by ozone-induced oxidative stress. Maize inbreds (n=50) and hybrids (n=26) were grown under ambient (~40 ppb) and elevated (~100ppb) [O3] at the FACE (Free Air Concentration Enrichment) site in Savoy, IL. All primary ears were harvested from each 11' hybrid row, and primary ears from eight contiguous plants harvested from each inbred row. All ears were phenotyped for length, diameter, row number, and kernels per row.

Hybrid ear length and diameter were significantly reduced under elevated ozone compared to ambient conditions, with B73xMo17, B73xCML333, B73xHp301, and B73xKi3 hybrids most affected. Inbred ear length was also significantly reduced under elevated ozone. Ear row number was not affected by ozone treatment, and the number of kernels per row was only marginally reduced. These results suggest that elevated O3 affects maize ear architecture primarily during grain filling, after the total number of kernels has been determined.

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P247

Effects of Planting Density on Vegetative and Reproductive Development in Adapted and Unadapted Populations of a Recurrent Selection Program

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Iowa Stiff Stalk Synthetic, BSSS(R), and Iowa Synthetic Corn Borer No. 1, BSCB1(R), have undergone 17 cycles of a reciprocal recurrent selection program with primary emphasis resting on increasing grain yields. Previous studies have shown that selection for grain yield has also produced germplasm that is increasingly adapted to higher planting densities. While much is known about the effect of planting density on final yields and plant phenotypes, the effect of density has been much less studied in regards to early plant and ear development, especially pre-silking. The objective of this experiment was to examine the effects of high planting density on the development of the reproductive and vegetative structures in adapted and unadapted BSSS(R) and BSCB1(R) populations. The cycle 0 base populations and the cycle 17 populations were planted at four densities ranging from 3.23 to 12.92 plants/m^2 at three locations around Ames, IA over the course of two years. High planting density reduced growth rate and delayed development of ears, tassels and shoots. High planting density reduced the rate of increase in ear length in BSCB1 but not in BSSS, and delayed ear and tassel mass accumulation in BSSS but not in BSCB1. The delays in ear and tassel mass accumulation observed with high planting density in BSSS were greatly reduced by selection in the cycle 17 populations.

P248

Efficiency of QTL Mapping in Popcorn Using Bayesian Approach

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Popcorn is an important food crop and popping phenomenon of popcorn is largely governed by the pericarp thickness and moisture in the kernel. while production of large flakes is associated with the ratio of hard to soft endosperm. Markers on the genetic maps are genes with variant alleles producing detectably different phenotypes. Objective of the study was to generate a simulated data of two sample sizes of 200 and 400 individuals of inbred lines and to identify efficiency of Bayesian approach for quality traits loci mapping. Genetic linkage map comprising of 10 linkage groups was constructed using 100 simple sequence repeat (SSR) markers. A total of 12 estimated QTL of major effect were detected. One QTL was determined on each chromosome 1 and 3 while 2 QTL were detected on each chromosome 6 and 7, while 3 QTL were found on each chromosome 5 and 10 of the genetic linkage map. Estimated 12 QTLs explained approximately 25 and 50% of the phenotypic variance for the heritabilities 30% and 70%, respectively. That is, each QTL explained approximately 2 and 4% of the phenotypic variance for the heritabilities 30% and 70%. Simulated data was used for power of QTL detection1 (%) and Bias1 (cM) with two sample size 200 and 400 individuals, two heritability's 0.3% and 0.7% through R program using Bayesian approach. We determined the power of QTL detection1 (%) and average number of false QTLs in chromosomes with none, one and 2-3 true QTLs and Bias1 (cM) between estimated and true position of identified QTLs using R package eqtl and qtlbim. Results show high power of QTL detection1 (%) and average number of false QTLs with 400 individuals and 0.7 h2 regardless of the trait in place of 200 individuals and 0.3 h2. The results for Bias1 (cM) between estimated and true position of identified QTLs show close similarity with Power of QTL detection. We concluded that increasing the population size and h2 , will increase the power of QTL detection and Bias1 (cM) between estimated and true position of identified QTL and vice versa. Present research work will provide important basic knowledge in the area of mapping molecular markers and analysis of quantitative trait loci for quality in popcorn.

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P249

Estimating ozone sensitivity in diverse maize germplasm with hyperspectral reflectance spectroscopy

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Tropospheric ozone (O_3) is one of the most harmful air pollutants impacting crop performance, causing an estimated $500 million in annual U.S. maize production losses. To investigate the response of maize to O_3, we screened 30 hybrid and 200 inbred genotypes, including the nested association mapping population, in ambient (~40 ppb) and elevated (100 ppb) [O_3] at the Free Air Concentration Enrichment field site in Champaign, IL. In general, growth in elevated [O_3] caused an increase in leaf senescence and a decrease in yield parameters, although significant genetic variation was observed. Hyperspectral reflectance spectroscopy (HRS) measurements were also taken to test the accuracy of using leaf optical properties to predict physiological traits, including soluble sugar content and maximum photosynthetic capacity (V_{max}). Using a subset of plants, HRS measurements were compared with standard methods to generate a predictive model using Partial Least Squares Regression (PLSR) analysis, resulting in a correlation coefficient (R^2) of 0.79 for foliar sucrose and 0.62 for V_{max}. We then applied the PLSR model to HRS measurements from all experimental rows, which consisted of 1024 inbred and 512 hybrid plants, once during vegetative and reproductive (12 days after anthesis) growth. Many genotypes were found to have O_3-mediated reductions in foliar sucrose and V_{max}. For future research, these and other analyses will be used to select a sensitive and tolerant genotype as parents for developing a recombinant inbred population to identify quantitative trait loci related to O_3 tolerance.

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

P250

Fall armyworm herbivory affects silica accumulation in corn and rice

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Plants, especially grasses, accumulate large amounts of silicon dioxide in their leaves. Silica deposition increases the strength and plant rigidity, but it is also a critical component of defense against both abiotic and biotic stresses including herbivores. This research determined the effect of plant silica deposition on insect herbivory and its inducibility. We used corn, rice and the fall armyworm (FAW), Spodoptera frugiperda (Lepidoptera: Noctuidae) as a model system. The inducibility of silica was tested by exposing the plants to herbivory for 24 hours and measuring the Si accumulation in the new regrowth tissues using the Molybdenum yellow method. The effect of silica on FAW mandible durability was evaluated by examining the mandibles of caterpillars fed on corn, rice and artificial diet using backscatter X-ray spectroscopy. Our results show that FAW herbivory induces silica accumulation in rice plants by ~37.2 % compared with undamaged controls. Furthermore, feeding on plants with high silica content destroys most of the mineralized area of the FAW mandibles (mainly composed of Zn, Na and Cl). We conclude that silica accumulation is an inducible plant defense mechanism that causes wear on the insect mandibles and likely hampers their ability to feed on plant tissues.

Funding acknowledgement: United States Department of Agriculture (USDA), Penn State University College of Agricultural Sciences, The Entomological society of America and Monsanto

P251

Fighting ROS and Aging Related Diseases

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Reactive oxygen species (ROS) are associated with a number of aging related diseases in humans, such as cancer, Alzheimer's Disease, and cardiovascular disease. Research suggests that diets rich in phenolic antioxidants may help prevent the onset of these diseases by preventing oxidation damage caused by ROS. However, a multitude of cross-sectional studies conducted in developed countries, including the United States, showed that consumption of fruits and vegetables traditionally recognized for containing high levels of phenolic antioxidants follows a socioeconomic gradient. Low socioeconomic status groups, defined by lack of education and income, tend to consume cheap, highly processed, and energy-dense foods rather than fruits and vegetables containing phenolic antioxidants. However, grains, especially maize, are known to possess high amounts of phenolic antioxidants. Furthermore, grains are key ingredients in processed snack foods and ready-to-eat cereals. As a first step toward improving the phenolic antioxidant content of maize-endosperm based foods through breeding, the variation in type and quantity of phenolic antioxidants such as ferulic acid, p-coumaric acid, and sinapic acid were examined in elite U.S. maize germplasm typical of that used in the production of maize-endosperm based foods.

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P252

From Genomic Regions to Individual Genes: Exploring the Genetic Architecture Underlying Seed Size Variation

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Seed size is an important grain yield component trait and positively correlated with grain yield. Previously, selection mapping in the Krug Yellow Dent long-term selection experiment and association mapping in the maize nested association panel were used to identify regions of the genome potentially controlling seed size variation in maize. However, these candidate regions often contain multiple annotated gene models. To transition from genomic regions to individual genes, differential gene expression analysis was used to refine the previously identified regions and identify candidate genes affecting seed size variation. Evaluation of endosperm tissue at 12, 15, and 18 days after pollination in large and small seeded genotypes revealed differential expression of 639 out of 3,037 genes within the candidate regions. Candidate genes are currently being validated using mutant stocks from the maize UniformMu resource. Current results support hypotheses for functional roles of diverse genes in determining seed size and demonstrates marked progress in the transition from genomic regions to individual genes.

Funding acknowledgement: DuPont Pioneer, Minnesota Corn Research & Promotion Council

P253

Functional characterization of ssp. *mexicana* introgression to Mexican highland maize: A possible role in local adaptation

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Maize was domesticated ca. 9,000 years ago in southwest Mexico from the lowland teosinte taxon *Zea mays* ssp. *parviglumis* (henceforth *parviglumis*). Following domestication, maize spread to the highlands of central Mexico, where it encountered dramatically different environmental conditions and came into sympatry with a highland teosinte ssp. *mexicana* (henceforth *mexicana*). Modern highland maize and *mexicana* share multiple phenotypic traits (e.g., abundant macrohairs and leaf pigmentation) that have been attributed to *mexicana* introgression into maize. At the genome level, it is estimated that ~20% of the genetic content of highland maize is derived from *mexicana*. Mapping of *mexicana* introgression has identified several regions that are shared across highland maize populations. Interestingly, shared regions of introgression were found to co-localize with previously identified quantitative trait locus (QTL) for highland traits, suggesting a possible role for *mexicana* in conferring maize adaptation to highland conditions.

To evaluate the phenotypic effects of *mexicana* introgression, we are generating near-isogenic test-stocks using marker-assisted backcrossing to introduce candidate genomic regions from highland maize and *mexicana* into the reference maize line B73. We will present advances in stock generation and describe our plans to characterize these materials in a variety of Mexican lowland and highland field sites.

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

P254

Genetic Analysis of White Core and Grain Size of "Yamadanishiki", a Japanese-sake brewing cultivar.

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Japanese rice wine (sake) is made from brewing rice. Sake is brewed from steamed rice through the fermentation by koji (*Aspergillus oryzae*) and yeast (*Saccharomyces cerevisiae*). A brewing rice cultivar, "Yamadanishiki" is the most popular in Japan. Several characteristics of brewing rice are large grain and presence of white core, which is opaque in the central rice grain, and suitable for high-grade polishing of rice grain, fast water absorption and amylolysis efficiency. To develop novel brewing rice, we need their genetic information. In this study, we carried out QTL analysis using F2 and RILs derived from Koshihikari/Yamadanishiki. Koshihikari is the leading cultivar in Japan. In addition, we measured the sequential growth rate of grains in both parents. We are going to discuss the development of white core and large grain based on the detected QTLs and the growth rate of Yamadanishiki grain.

Funding acknowledgement:

P255

Genetic architecture of phenotype-selected introgression families (PIFs) in maize

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Introgression of exotic germplasm can enhance the genetic variation of elite maize breeding material and ensure long term improvement of agronomically important traits. This study aimed at developing and characterizing two maize introgression libraries for a collection of dominant PHT - increasing alleles by introgressing donor chromosome segments (DCS) from Germplasm Enhancement of Maize (GEM) lines into elite line PHB47, PHZ51. Collectively, these phenotypically selected introgression families (PIFs) are comparable to introgression line libraries, with two major differences: (1) a complete set of PIFs for PHT does not necessarily cover all regions of the genome, but accumulates chromosome segments affecting PHT; and (2) the introgressed donor segments originate from multiple donors. Different backcross families (BC1- 4) were developed and the tallest 23 families from each introgression library were selected for single nucleotide polymorphisms (SNPs) genotyping to localize DCS underlying plant height. The result shows that most PIFs carrying DCS were significantly ($P = 0.01$) taller than their respective recurrent parent. What's more, they were comprised with a larger donor genome proportion compared to a theoretical proportion without selection or random mating across BC1-4 generations. These PIFs contained 3 to 15 heterozygous DCS, which were distributed over the whole genome in the two selected libraries, indicating the complex genetic nature underlying this trait. We conclude that our PIFs containing favorable PHT-increasing alleles; these two libraries may offer opportunities for future PHT allele mapping process or for breeding perspectives.

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P256

Genetic dissection of the seedling root traits using ultra-high density bin-map in a maize recombinant inbred line population

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The maize root system mediates key functions of anchorage and acquisition of nutrients and water. In contrast to above-ground plant architecture, root systems also exhibit much greater capability to respond to their environment. Nevertheless, relatively few genes controlling this important aspect of plant architecture have been identified in maize. Genetic mapping using bi-parental crosses is a powerful tool for uncovering influential loci for traits of interest.In our present study, a 230 RIL subset from a large-population derived from the widely adapted Chinese hybrid Zhengdan958(Zheng58×Chang7-2), was evaluated as seedlings for lateral root density (LRD), lateral root number (LRN), primary root length (PRL), total root length (TRL), seedling dry weight (SDW), root dry weight (RDW) and root to seedling ratio (RSR). A high density bin map comprised of 7319 bin markers derived from genotyping by sequencing (GBS) was used to construct the genetic map for 230 recombinant inbred lines. The quality of the bin map was verified by the co-localization of the reported gene P1 (pericarp color1). Based on the map, a total of 18 QTLs were located to the nine out of ten chromosomal regions with bin marker intervals ranging from 1.4 Mb to 52.2 Mb. qLRN1 was located to the short arm of Chromosome 2, and is co-located with the mutant lrt1. Furthermore, three QTLs of qLRD2 (2.04), qLRN3 (6.05) and qPRL(2.08) were co-locate with the reported root-QTL clusters. These results provide potential QTLs for genetically improving the seedling root architecture traits, and the insight into the contributions of the elite parents Zheng58 and Chang7-2 to the embryonic root system of ZD958.

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P257

Genetic relationships among Guelph and off-PVP maize lines

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Genotyping-by-Sequencing (GBS) is a popular method of genotyping a large number of SNP markers at low cost. GBS data for 110 Guelph maize (*Zea mays*) inbred lines was merged with publically available GBS data for 2,765 inbred lines from panzea.org. SNPs were filtered for minor allele frequency ≥ 0.05 and minimum call rate of 10%, with 379,578 SNPs (39.7%) passing filtering criteria. A network diagram of genetic similarity was created using the network visualization software Gephi and a genomic relationship matrix. This analysis shows off-PVP lines clustering into Stiff Stalk (B73, B14 unrelated SS), Iodent (PHP207) and Lancaster (Mo17, Oh43). Guelph lines show similarity to the PHP207, B14 and unrelated SS clusters and form a novel cluster including lines with Wf9 and Mn13 backgrounds. We propose that GBS data can be used to create a genomic relationship matrix, describing the relationships of inbred lines, which can then be used for generating IBD haplotypes, when complete pedigrees are not available. We are interested in using Identity-by-Descent (IBD) haplotype data and breeding values generated from a NC Design II experiment to fit a mixed linear model to the data and identify QTLs associated with grain yield, response to plant density, and environmental stability.

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P258

Genome wide association for flowering time in a comprehensive panel of maize landraces

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Landraces represent an unprecedented source for useful alleles for maize breeding, however access to these alleles is hampered by poor performance, lack of adaptation and lack of information. Until recently, the identification and deployment of high value alleles has been slowed by their linkage with numerous undesirable alleles and the lack of phenotypic association with genetic markers.

Here, we present the results of performing Genome Wide Association for flowering time in a landrace panel of 3,500 landraces from CIMMYT, representing the diversity of 36 Latin American countries. Flowering time is a trait critical for local adaptation and therefore a major barrier for the exchange of alleles across gene pools. In addition, it has been extensively characterized in elite materials and as such offers an excellent model to evaluate genome wide association approaches employing landrace germplasm. We performed phenotypic evaluation in 23 trials across Mexico for 2 years, and using Genotyping by Sequencing, we generated around 1 million SNPs. Using linear mixed models, we identified significant association between flowering time and genes known to be part of its genetic architecture, validating the experimental approach adopted. Furthermore, we report additional association of flowering time with natural standing variation at previously unreported genes. Finally, we report significant association between flowering time and structural variation, including large genomic inversions and centromeres. We further show the high predictive ability of the significant loci in genome wide prediction models.

This study represents the largest survey of landraces to date, and provides an experimental design appropriate to evaluate alleles from exotic germplasm in a large scale. It also provides new knowledge about the genetic basis of an agronomically and evolutionary important trait, and provides evidence for a major role of large structural variation as part of the genetic architecture of quantitative traits in maize.

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P259

Genome-Wide Association Analysis of Tassel Size and Branch Number in the Wisconsin Diverse Association Panel

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Maize tassel size is important in determining the timing and amount of pollen available for hybrid seed production as well as within agricultural production fields. Tassel size may also be negatively associated with yield due to shading and carbon partitioning. In this experiment, we measured tassel characteristics in the Wisconsin Diverse (WiDiv) panel of inbreds and conducted genome-wide association analysis. Tassel parameters measured included tassel length, branch number, spike length, spike proportion, and branch zone. Heritabilities for all five traits ranged from 0.87 to 0.93. Tassel branch number ranged from 0 to 38 and was negatively associated with year of inbreds' release ($R^2 = 0.25$), consistent with selection for high grain yield reducing tassel size. GWAS of 555 lines using 538,567 SNPs from RNA-sequencing and 681,257 SNPs from the Ames panel data set identified a significant association on chromosome 7 with the most significant SNP in GRMZM2G178522. This gene is near a previously characterized gene affecting spikelet pair production, *ramosa1*. Additional detail on this candidate gene and related candidates from the literature will be described. This study provides new information on the control of natural variation for floral structures in maize, and serves as a model for the utility of the WiDiv panel and RNA-sequencing-based SNP data set to identify genes underlying natural variation in maize.

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P260

Genome-Wide Association Study of Domesticated Traits in Maize

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Maize (corn) was domesticated about 8000 years ago from a wild grass, teosinte. Numerous morphological traits have changed in maize compared to its wild ancestor, including the floral morphology. Teosinte produces flowers on many nodes along lateral branches, with each flower having female florets near the base and terminating in male tips. In contrast, maize normally has male flowers (tassels) only at the top of the main stem of the plant, and has usually only a single completely female ear at the end of a highly reduced lateral branch called a shank. Furthermore, teosinte flowers produce one row of seeds, whereas modern maize can produce ears with more than 20 rows of seeds. Several major QTL and specific genes controlling these differences between maize and teosinte have been identified(Studer & Doebley, 2012; Wills et al., 2013). Despite the severe bottleneck that occurred during domestication and strong selection for the maize plant type, these traits still vary among different maize varieties. To uncover the underlying genetics basis for the phenotypic variation, genome-wide association has been conducted on 2480 maize inbred lines and Nested Association Mapping populations. It reveals several significant association among the whole genome.

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P261

Genomic Prediction in maize (Zea mays L.) based on seedling root length in an effort to use root phenotypes as a selection criterion in plant breeding.

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The maize root system is crucial for plant establishment as well as water and nutrient uptake. Root systems have been found to be plastic by nature and there is substantial genetic and phenotypic variation for root architecture, giving an opportunity for selection. However, root traits have not yet been used as selection criterion due to the difficulty in collecting phenotypic data. Genomic prediction (GP) could allow one to phenotype only in a training population and use root traits as a selection criterion for plant breeding. A Ridge Regression BLUP (RR-BLUP) strategy was used to train a GP model. A training population made up of 384 inbred lines from the Ames Panel was phenotyped by extracting root traits from images using the software program ARIA. BLUPs were predicted for the remaining 2400 inbred lines based on the trait Total Root Length (TRL). Selections were made on the 100 genotypes with the longest and 100 shortest predicted roots to act as a validation population.

Cross-validation found an average prediction accuracy based on a Pearsons correlation between predicted TRL and observed TRL of r=0.4204. Prediction accuracy for all 200 inbred lines within the validation population was found to be r= 0.594. Within each grouping, 100 long predicted roots and 100 short predicted roots, prediction accuracy was much lower at r=0.102 and r=0.12 for each group respectively. For breeding purposes, ranking genotypes accurately is more important than predicting the exact length of a root system. Root length ranking accuracy found using a spearman correlation between the predicted ranking of genotypes based on length the ranking observed was found to be ρ=0.546. These results show GP is a promising method of using roots for selection. Field studies need to be conducted to determine how hydroponic seedling studies relate to root growth in the field.

P262

Genomic Prediction in NAM and the Ames Inbred Collection

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The accuracy of genomic prediction in maize depends on a variety of factors, which include population structure and the relatedness of the training population to the population to be predicted or target population. When the training and target populations are not closely related prediction accuracy can be poor. The inclusion of prior information such as QTL or genomic annotations should help improve prediction accuracy. This study describes the establishment of baseline genomic prediction models in two populations, the Nested Association Mapping (NAM) population and the Ames inbred collection. The baseline models do not contain information from priors but will provide a basis for assessing any improvement made by incorporating additional information. To aid with that assessment the study will identify training and test sets that provide a range of accuracies.

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P263

Genomic Prediction of Hybrid Performance in Maize (*Zea Mays L.*)

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Prediction of hybrid performance is extremely important as it is difficult to evaluate all possible single cross combinations between inbred lines. Genomic prediction of hybrid performance has shown to be promising approach through simulation and experimental studies. However, only limited studies examined the accuracy to estimate the hybrid performance, while most studies rather focused on predicting general combining ability (GCA) as one component of hybrid performance. Our objectives were to 1. Examine the prospects of genotyping by sequencing for predicting hybrid performance in maize, 2. Compare the accuracy of genomic predictions for hybrid performance within and across families when the families are related and 3. Evaluate the effect of marker number, training population size, composition of training set and genomic selection models on prediction accuracy. We used 312 maize single cross hybrids made between 46 female and 171 male recombinant inbred lines or doubled haploid lines. The hybrids were belonging to nine families related through common parents. The hybrids were evaluated for grain yield in a multi-location experiment during 2012-13. All 217 lines were genotyped with genotyping by sequencing (GBS) approach. Hybrid performance was predicted by genomic best linear unbiased prediction (G-BLUP) including additive and dominance relationship matrices. We observed in cross validation a prediction accuracy of 0.70 for across family hybrid prediction. Interestingly, modelling dominance did not increase prediction accuracy. When GBS markers with more than 25 % missing data were included in the analysis, a slight decrease in prediction accuracy was observed. The prediction accuracies for within-family hybrid prediction were moderate to high, illustrating the potential of genomic prediction to estimate hybrid performance within a family when the training set consist of hybrids from other related families. Overall, our results suggest that genomic prediction of hybrid performance holds good potential to increase the efficiency of hybrid breeding.

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P264

Genomic Prediction of Ionomic Traits in the Maize Nested Association Mapping Panel

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Mineral nutrient deficiencies, especially iron and zinc, are global health problems that are strongly associated with poverty and food insecurity. Developed countries have greatly reduced such deficiencies through dietary diversification, food fortification, and supplementation. In developing countries, however, these strategies are often too expensive, inaccessible, and/or difficult to sustain. In these contexts, the genetic improvement of mineral content in the edible portion of staple crops such as maize, termed biofortification, offers a cost-effective and sustainable approach to help alleviate these deficiencies. The population size, genetic diversity, and multi-location trials of the US maize nested association mapping (US-NAM) panel provide a robust genomic and breeding platform to dissect the genetics underlying grain mineral nutrient concentrations. Ionomic profiles quantifying 20 elements (e.g. Fe, Zn, Se, and K) were generated on inductively coupled plasma-mass spectrometry for NAM recombinant inbred lines grown at four locations (FL, NC, NY and PR). Our statistical analysis returned thousands of SNPs associated with ionomic profiles both within and across the four locations. Statistical models commonly used in genomic selection were evaluated across this 20-trait ionomics data set to further understand the rate of genetic gain that could be achieved for grain mineral concentration using marker-based breeding approaches. The prediction accuracy of several statistical models for marker sets with different levels of genome coverage was examined because these traits are highly influenced by the environment and likely to have contrasting genetic architectures. The results of these tests in the context of the joint linkage-genome wide association study results will be presented.

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P265

Genomic prediction of provitamin A and vitamin E levels in maize grain

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Vitamin A deficiency, prevalent in many parts of Asia, sub-Saharan Africa, and Latin America, has been cited as responsible for 250,000 new cases of nighttime blindness and 600,000 early childhood deaths each year. Tocochromanols (vitamin E and antioxidants) are produced through a biosynthetic pathway partially shared with carotenoids (provitamin A) and also tend to be deficient in certain population segments including the elderly and immunocompromised. Improvement of provitamin A carotenoid and vitamin E tocochromanol levels in maize grain through breeding, a process termed biofortification, can particularly help in rural areas where diet diversification, fortified foods, and supplements are not easily accessible. Genomic prediction analyses in a panel of 281 diverse maize inbred lines found that markers targeting a small set of QTL identified in previous linkage analysis studies predicted grain carotenoid levels equally well as genome-wide markers, suggesting the viability of a carotenoid biosynthetic pathway-level breeding approach for orange, provitamin A-biofortified maize grain. Further prediction analyses are leveraging the substantially larger U.S. maize nested association mapping (US-NAM) panel to identify the statistical models and marker coverage and densities that maximize prediction accuracies and gains from selection for carotenoids as well as tocochromanols. Both sets of traits are candidates for marker-based selection approaches: costly to quantify on high-performance liquid chromatography and oligogenic with a clear biochemical basis, which may allow biological prior information compiled at the level of genes, transcripts, and metabolites to be used to improve predictions. In particular, assessment of the gains conferred by using priors from US-NAM, a panel in which variation was experimentally generated through crosses, to predict carotenoid and tocochromanol phenotypes in the 281 panel, compiled to represent worldwide natural variation, will assist in taking genomics results to the field for these nutritional grain traits of immediate interest to maize breeders.

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P266

Glossy15 as an insect resistance target for improvement of Mexican maize

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In Mexico, fall armyworm (FAW) remains the major pest of the maize crop, causing losses of up to 60% of total production. The first generations of insect larvae are the most damaging, feeding on the juvenile leaves of young plants. When maize plants are larger, they better withstand larval feeding damage. Previous mapping experiments using the FAW resistant line Mp708 have identified a resistance QTL linked to *Glossy15* (*Gl15*), a major regulator of juvenile-adult phase change. We hypothesize that a shortening of the juvenile phase resulting in an earlier expression of adult epidermal traits deters feeding by FAW larvae. To test this hypothesis, we are using molecular markers to introduce both the Mp708 QTL interval and the classical loss-of-function allele *gl15-Lambert* into the subtropical breeding line CML312. We will present current progress and outline our plans for functional testing in insect trials. In addition, we will comment on our broader efforts to identify further resistance sources in Mexican landrace maize and wild teosintes.

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P267

Heat Stress Tolerance in Maize

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Heat stress is a prominent and growing agricultural concern in many areas of the world, negatively impacting plant health and performance. It is estimated that 1% of grain yield is lost due to premature senescence and death of vegetative and reproductive structures for each day that the temperature rises above 30 °C (Lobell et al., 2011). In the maize-growing regions of South Asia, daily temperatures are regularly 40 °C and higher during reproduction and represent a significant constraint to plant growth. The Heat Tolerant Maize for Asia (HTMA) project is a Global Development Alliance funded by USAID to increase heat stress tolerance of maize with partners including CIMMYT, Purdue University and DuPont Pioneer as well as National Agricultural Research Systems and seed companies from South Asia. The HTMA project seeks to understand heat stress tolerance of maize at a physiological and genetic level and ultimately create superior hybrids that thrive under these conditions. A genome-wide association study (GWAS) was designed to reveal the underlying genetic controls of heat stress tolerance. The Heat-Tolerance Association Mapping (HTAM) panel includes temperate and tropical germplasm and exhibits contrasting responses to heat stresses. The panel was genotyped and extensively phenotyped under heat-stress and optimal conditions. Numerous phenotypes were collected including agronomic performance traits and leaf lipid composition. Preliminary analyses revealed multiple genetic loci that were associated with heat stress tolerance. The GWAS results will assist in identifying molecular markers that can be used by our HTMA partners for marker assisted selection (MAS) of heat-tolerant maize varieties.

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P268

Herbicide-safener GWAS for studying intraspecific variation in plant stress responses

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Plants are frequently exposed to stresses that elicit the rapid production and activation of defense mechanisms for adaptation and survival. Herbicide safeners are non-toxic compounds that confer herbicide protection to cereal crops by inducing detoxification systems, including massive increases in the activity of glutathione S-transferases (GSTs) and cytochrome P450s, through unknown mechanisms. Safener-induced gene expression confers protection from xenobiotics and stresses other than just the target herbicide, presumably by tapping into pre-existing, stress-responsive signaling pathways. The purpose of this experiment was to elucidate the molecular mechanisms underlying this process by performing a genome wide association study (GWAS) for herbicide sensitivity and safener response in a diverse panel of *Sorghum* inbreds. *Sorghum* inbreds (>800) were treated with both soil-applied herbicide (s-metolachlor) and safener (fluxofenim) (a), herbicide only (b), safener only (c), or left untreated (d). Total fresh weight (TFW), shoot number (SN), and fresh weight per shoot (FWS) were recorded for each treatment combination 12 days after planting. As expected, most inbreds were sensitive to herbicide in the absence of safener but grew well in the presence of both safener and herbicide. Other response classes included "constitutive resistance", in which safener treatment was not necessary to confer herbicide resistance, and "safener unresponsive", in which even safener treatment was not sufficient to rescue growth. Genome-wide association was performed for each of four single-treatment contrasts. Several significant GWAS hits mapped near GST genes previously shown to be upregulated in response to safener treatment. We propose that herbicide-safener screens provide a rapid and high-throughput means of studying early signaling in plant stress responses, and that understanding safener induction of endogenous detoxification pathways will provide opportunities to enhance abiotic stress tolerance in agricultural plants.

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P269

Heterosis of sorghum plant height caused by repulsion linkage in the *Dw3* gene region

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Heterosis contributes greatly to yield increase in many crop species. The mechanisms for heterosis, however, are still not clear. Many hypotheses have been proposed, including dominance, overdominance, pseudo-overdominance, epistasis, epigenetic changes, and protein metabolism changes. Here, we provide an example of heterosis in plant height generated by repulsion linkage, i.e. pseudo-overdominance. Using a sorghum recombinant inbred lines (RILs) population derived from Tx430 and P898012, a new quantitative trait loci (QTL) for plant height was identified 30 cM away from the *Dw3* gene on chromosome 7. Whenever the two QTL are in repulsion linkage and two parents have opposite alleles, the hybrid can show heterosis in plant height. This hypothesis was confirmed by observing plant height of F1 hybrids crossed from RILs with different allele combinations of the two QTL. Alleles conferring taller plant height at each QTL is complete dominant over alleles conferring shorter plant height, agreeing with previous studies. The results thus support the dominance hypothesis for heterosis. In contrast to *Dw3* gene, which does not have effect on the elongation of the top part of the stem, the linked QTL has effect on both higher and lower part of the stem, suggesting the new QTL regulates plant height in a way different from *Dw3*. The effect of this new plant height QTL is almost equal to other known plant height QTL in sorghum, making this QTL one of breeders' tools to manipulate plant height for grain or biomass production. This QTL was also detected using the Sorghum Diversity Panel (SAP) by including *Dw1*, *Dw2*, and *Dw3* genes as covariates, suggesting information from linkage mapping could guide association mapping to identify loci previously not detected.

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P270

Identification of nutrient –dense germplasm for maize biofortification

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Increasing human population and dwindling resources have put tremendous pressure on agriculture especially in Sub-Saharan Africa, Latin America and Asia. Of the Asian countries, India has made significant progress in improving food security of its masses. Although there has been surplus of food grains at national level, yet malnutrition persists. Maize with higher bioavailability of nutrients/micro-nutrients and better digestibility ie.quality protein maize (qpm), therefore, has a great potential in improving nutrition status especially of the poor masses. Hence, it became pertinent to have an insight on the Indian maize lines with potential for biofortification. Accordingly, a set of elite inbred lines comprising common maize (110), quality protein maize (55) and others (35 including sweet corn, pop corn and waxy) developed at different breeding stations of erstwhile Directorate of maize research, was grown under 10 environments (year x location combination) over five years (2008-2012) to identify superior inbred lines with high per se performance and improved nutritional profile so as to augment the development of stress-tolerant nutritionally enriched cultivars. A number of inbred lines of common maize as well as qpm were identified based on 1) phenotypic data :relative maturity (early, medium and late), kernel colour (yellow, orange and white) ,kernel texture (flint and dent), yield and yield-related traits;2) biochemical data :% protein, % lysine, % tryptophan, % oil, % starch; and micro-nutrients, viz.total carotenoids;3) molecular data : SSRs and,4) reaction to various fungal diseases at hot spot/ artificial epiphytotic conditions/ locations. The lines displayed >0.30<0.94 % tryptophan,> 1.21 <4.2 % lysine in protein, respectively, with protein (in mature endosperm kernels) >6.78 < 13.34 % and oil >3.0<5.58 %, respectively. A few yellow qpm lines displayed total carotenoids >31.0 ug/g. The generated data was statistically analyzed and interpreted in elucidating the breeding potential of the selected lines in augmenting the development of nutrient-dense cultivars in maize.

P271

Identification and Characterization of the Genetic Components of Apomixis in Maize (*Zea mays* L.)

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Apomixis, the asexual reproduction through seeds, occurs in more than 400 species belonging to about 40 plant families, but it is absent in major crops. The production of seeds though apomixis, which generates plants that are genetically identical to their mother, has an enormous agricultural potential to indefinitely maintain desired genotypes, *e.g.* the heterozygosity of F1 hybrids.

Gametophytic apomixis deviates from sexual development in three major steps that constitute the elements of apomixis: (1) meiosis is circumvented or aborted, leading to the formation of unreduced, unrecombined embryo sacs (apomeiosis); (2) embryogenesis initiates without fertilization of the unreduced egg cell (parthenogenesis); and (3) developmental adaptations allow the formation of functional endosperm.

To harness apomixis in crop plants, we have been searching for maize mutants displaying these individual elements of apomixis. Combining such mutations in one plant should result in apomixis and, thus, clonal reproduction. Here we summarize the results of two genetic screens for mutants displaying apomeiosis and parthenogenesis. The genetic screen for apomeiosis mutants was based on the ploidy barrier for endosperm development. Plants carrying active *Mu* transposons were pollinated by a tetraploid tester and the progeny was screened for plump kernels, as a read-out of unreduced female gametophytes. We will present a genetic, cytological, and functional characterization of the most promising mutant derived from this screen.

To identify the genetic bases of the second component of apomixis, parthenogenesis, we initiated another screen. W23 pollen carrying the *C1* and *R1-nj* alleles were subjected to EMS mutagenesis. M1 plants were pollinated by a W23 line homozygous for the *C1-I* allele, an inhibitor of *C1*, and resultant ears were screened for kernels with haploid embryos, which had showed anthocyanin pigmentation in the *R1-nj* pattern, indicating development of the embryo without paternal contribution. We will present first results of this screen.

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P272

Identification of a modifier of Oy1 (moy1) with the ability to boost photosynthesis

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Natural variation in the germplasm is fundamental for any crop improvement strategy. Utilizing information from this genetic resource for the identification and characterization of the genetic components underlying natural variation is of paramount importance. We conducted a Mutant-Assisted Gene Identification and Characterization (MAGIC) screen using the Oil yellow-1 (Oy1) mutant as a reporter to identify potential genomic regions that are involved in enhancing photosynthetic activity in maize through chlorophyll biosynthesis. The Oy1 maize mutant is defective in subunit I of the magnesium chelatase enzyme, involved in catalyzing the first committed step of chlorophyll biosynthesis. The Oy1 allele used in this screen was semi-dominant and had been introgressed into B73 over seven generations. To detect modifiers of Oy1, pollen from an Oy1 heterozygote (Oy1/+::B73) was crossed with a collection of maize inbreds, including NAM founders. As expected, some of the inbreds were found to enhance the phenotype while others suppressed it significantly. Interestingly, Mo17 was one of the most enhancing inbreds identified. Besides affecting the chlorophyll content of the leaves, differences in severity of the Oy1 phenotype were directly related to plant vigor. In addition, it also had a direct bearing on the maturity level of the plant. A MAGIC screen of the IBM population, allowed us to identify a QTL that maps to the same region on chromosome 10 where Oy1 is located. It appears that this modifier of Oy1 (dubbed moy1) has the ability to modulate chlorophyll biosynthesis and, as a result, photosynthesis. This study also unveils a new maturity-regulating mechanism in corn.

Funding acknowledgement: National Science Foundation (NSF)

P273

Identification of candidate genes associated with carbon isotope discrimination and drought tolerance in maize

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Drought is one of the major constraints of plant productivity worldwide. Understanding genetic and molecular mechanisms underlying drought tolerance is required for genome based breeding of improved cultivars with higher yield and yield stability under adverse climatic conditions. We investigated genetic mechanisms underlying complex traits hypothesized to be important components of drought tolerance such as stable carbon isotope discrimination ($\Delta^{13}C$) and stomatal conductance (*Gs*) in maize. The relevance of $\Delta^{13}C$ as an indirect trait for selecting drought tolerant varieties is well established in C_3 plants. However, knowledge about the genetic architecture and control of $\Delta^{13}C$ in the C_4 plant maize and its association with drought tolerance is limited. In order to identify genetic components of $\Delta^{13}C$ in maize, we made use of a maize introgression library consisting of 89 introgression lines derived from a drought tolerant *dent* line as recurrent parent and a *flint* line as donor parent (Gresset et al., 2014). For candidate gene identification we focus on a target region on chromosome 7, which accounts for 15% phenotypic variance for $\Delta^{13}C$. In parallel we make use of next generation sequencing techniques to gain insights into the maize transcriptome under drought of the two parental lines. The integration of the two approaches shows that candidate genes identified for ^{13}C also play a role in drought tolerance.

Funding acknowledgement: DFG

P274

Improving Standards and Methods for Phenotypic Prediction

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Genotypic, phenotypic, and environmental data must be documented, organized, and analyzed to enable the breeding decision-making process and to support the emerging field of phenotypic prediction. Many data types collected for these purposes have multiple standards for collection and data representation while others have no existing standards documented. This creates downstream difficulties for data integration and analysis – especially for multi-location projects that involve a large number of research groups. To address this need, we have partnered with the GXE subgroup of the Genomes to Fields (G2F) Initiative to develop data management protocols, standards, and systems that meet not only their data management needs, but that generalize to the broad community of maize breeders and geneticists. Here we describe our progress toward deploying data management and analysis solutions for maize predictive phenomics and breeding.

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P275

Insights from Classic Diallel Design into GWAS, GS, and Heterosis

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A diallel involves crosses from all possible combinations of parents, which is commonly used to estimate general and specific combining ability and their corresponding variances. With the next generation sequencing (NGS) technology, genomewide high density molecular markers become available at a low cost. Integrating the classic diallel design, high quality phenotype, huge quantity of marker data, and statistical algorithms can bring new sights into quantitative genetics, enabling us to better understand QTL detection, genomic prediction, and heterosis mechanism. With 24 diverse founder inbreds from nested association mapping, 276 single crosses were generated according to partial diallel design. Using mixed model considering general and specific combing ability together, we identified several loci significantly associated with plant height, days to anthesis, and grain yield. We further predicted hybrid performance in different training population schemes. The constructed prediction model can be applied to hybrid breeding programs by evaluating grain yield potential of hybrids before field trials. Heterosis is less related to number of heterozygous loci (determination coefficient R^2= 0.01~0.09), but more related to number of homozygous loci with favorable alleles (R^2 = 0.17 ~ 0.58). Loci with dominance degree ($|d/a|$) less than 1 contributed enormously to hybrid performance for all traits. The results indicate that genetic basis of heterosis can be explained primarily by accumulated genetic effects. Our findings suggest that high density markers across whole genome allow classic diallel to answer complex and challenging genetic questions.

P276

Investigation of Several Critical Questions in Genomic Selection

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Genomic selection is a procedure that foregoes significance tests and uses a large set of random markers for marker-based selection. Recent advances in next generation sequencing technologies and statistical algorithms make genomic selection feasible for plant breeders. When applying genomic selection to plant breeding programs, there are several critical questions that need to be addressed: *1) Which prediction model should be used? 2) How to perform cross validation to assess the predictability of the model? 3) Will population structure impact the prediction?* Here, we investigated these questions in two plant populations: a set of 277 diverse maize inbred lines with complex familial relationship and population structure, and 1,000 globally collected biomass sorghum accessions. We first compared the genomic estimated breeding values (GEBVs) and prediction accuracy from six common genomic selection models: ridge regression best linear unbiased prediction (RR-BLUP), exponential kernel, Gaussian kernel, BayesA, BayesB, and BayesCπ. Second, we evaluated three cross validation schemes: *k*-fold cross validation, repeated random sub-sampling cross validation, and leave-one-out cross validation (i.e., Jackknife). Finally, we demonstrated the impact of population structure on genomic selection. With a good understanding of these questions, we can further focus on optimization of training set, validation set, and testing site to tackle questions such as prediction in the context of broad germplasm and genotype by environment interaction.

Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE)

P277

Joint Analysis of European Nested association Mapping Populations Reveals Different Multiallelic QTL for Hybrid Performance in the Flint and Dent Heterotic Groups of Maize

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Multiparental designs combined with dense genotyping of parents have been proposed as a way to increase the diversity and resolution of quantitative trait loci (QTL) mapping studies, using methods combining linkage disequilibrium information with linkage analysis (LDLA). Two new nested association mapping designs adapted to European conditions were derived from the complementary dent and flint heterotic groups of maize (Zea mays L.). Ten biparental dent families (N = 841) and 11 biparental flint families (N = 811) were genotyped with 56,110 single nucleotide polymorphism markers (Bauer et al., 2014, Genome Biology) and evaluated as test crosses with the central line of the reciprocal design for biomass yield, plant height, and precocity. Alleles at candidate QTL were defined as (i) parental alleles, (ii) haplotypic identity by descent, and (iii) single-marker groupings. Between five and 16 QTL were detected depending on the model, trait, and genetic group considered. In the flint design, a major QTL (R2 = 27%) with pleiotropic effects was detected on chromosome 10 in the ZmCCT region. It could be related to the presence of a late allele specific to north-central European materials. Other QTL displayed milder effects (R2 < 10%). On average, the LDLA models detected more QTL but generally explained lower percentages of variance, consistent with the fact that most QTL display complex allelic series. Only 15% of the QTL were common to the two designs. A joint analysis of the two designs was conducted considering that the Central Dent - Central Flint genotype segregated in both designs. It detected between 15 and 21 QTL for the five traits. Of these, between 27 for silking date and 41% for tasseling date were significant in both groups. Favorable allelic effects detected in both groups open perspectives for their reciprocal selection to improve biomass production.

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P278

Joint-Linkage QTL analysis for total kernel protein content in teosinte near isogenic lines

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Teosinte (*Zea mays* ssp. *parviglumis*) is the wild ancestor of modern maize (*Zea mays* ssp. *mays*). Teosinte contains much larger genetic diversity compared to maize inbreds and landraces, but is limited by genetic resources to evaluate its value. A population of teosinte near isogenic lines (Teo-NIL) was developed to broaden the resources for genetic diversity of maize, and to discover and evaluate novel agronomic and domestication traits. The 928 Teo-NILs were developed by backcrossing ten geographically diverse *parviglumis* accessions into the B73 background. Each Teo-NIL has an average of 2.4 centimorgan (cM) of chromosomal segments from teosinte genome introgressed into B73. In this study, we calibrated a FOSS® 6500 Near Infrared Reflectance Spectroscopy (NIRS) instrument to predict total kernel protein content on a dry matter basis in bulk whole grains of 697 Teo-NILs that were grown in one replicates in two environments. We then conducted a joint-linkage quantitative trait locus (QTL) mapping study of kernel protein content in the NIL population. The joint-linkage QTL analysis revealed two (novel) QTLs with a range of strong allelic effects for kernel protein content. Our results strongly support our hypothesis that teosinte does harbor novel alleles for kernel composition and can be exploited for the improvement of kernel traits in modern maize germplasm.

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P279

Leaf-level hyperspectral reflectance as a tool for measuring photosynthetic capacity in C4 grasses

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Modern genomic and genetic tools are not being fully leveraged to improve the photosynthetic capacity of food and fuel crops due to a phenotyping bottleneck. Currently available techniques for measuring photosynthetic performance are not capable of both accurately estimating photosynthetic capacity and achieving the required high-throughput for phenotyping a population. Recently, optical, near-surface reflectance spectroscopy has been proposed and tested as a **method for high-throughput screening** of photosynthetic capacity in C_3 trees and crops. This approach was tested during the summer growing seasons of 2013 and 2014 in C_4 grasses using a family of 180 phenotypically diverse recombinant inbred lines (RILs) resulting from a cross of *Setaria viridis* and *S. Italica*. Photosynthetic capacity measured by leaf gas exchange and leaf reflectance spectra (350-2500nm) varied significantly across a subset of the population. Partial Least Squared Regression is being used to model the relationship between photosynthetic capacity and leaf optical properties. This model will then be applied to spectra measured on all 180 RILs in 4 different environments (high planting density, low planting density, well-watered, and water-limited) in order to test genotype by environment interactions in quantitative trait loci mapping of photosynthetic capacity in this model C_4 grass.

Funding acknowledgement: Department of Energy (DOE)

P280

Maize on the spot: Getting to the bottom of leaf lesions, flecking and spotting

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We have been analyzing the genetic bases controlling natural variation in several phenomena that cause lesions or flecking on maize leaves. These phenomena include resistance to leaf blight diseases, Southern leaf blight, Northern leaf blight and Gray leaf spot; variation in the severity of lesions in a maize autoimmune mutant and in a 'natural flecking' phenotype observed in many maize lines. We will present our latest data and hypotheses, including data on possible connections between the genes and metabolic pathways that control these traits and on the possibility of using flecking as a selection target to improve broad spectrum resistance.

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

P281

Mapping and identification of increased protein digestibility in sorghum

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Unlike corn, sorghum becomes much less digestible after it is cooked, presenting major nutritional challenges to hundreds of millions of people who rely on it as a staple food. Shortly after the high lysine maize mutant opaque2 (o2) was discovered and characterized, work began on developing a high lysine sorghum cultivar. Similar to o2 mutants, which are deficient in prolamin (zein) production in the seed, the high lysine sorghum mutant, P721Q, has recently been shown to have a defect in a gene for one of its prolamins (kafirins) [Wu et al 2014]. In addition to high lysine, P721Q also exhibits a 3-4-fold increase in protein digestibility after cooking, which makes it even more valuable as a food staple. However, it is not clear that the kafirin mutation also causes the improved protein digestibility of this mutant, and interesting preliminary data have suggested it may not; rather, this improvement may be caused by a mutation in a separate gene that has not yet been linked to protein digestibility. Mapping the mutation that causes increased digestibility in the P721Q mutant is presented.

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P282

Mapping and identifying candidate genes of the *modifier of amylose extender 1* (*mae1*) mutation in maize (*Zea mays* L.)

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Knowledge of the genes and proteins involved in starch synthesis has grown tremendously by the study of reverse genetics, however the complex process is still not fully understood. In maize (*Zea mays* L.), *amylose extender 1* (*ae1*) mutants are associated with an increase in amylose-like starch from 25 to 70%, affecting kernel hardness and levels of resistant starch. A novel maize phenotype of completely shrunken, collapsed kernels was observed when backcrossing two recessive *ae1* alleles into the white food-grade inbred line cgx333. This novel mutation appears to be recessive and due to the segregation of a single gene, which we have named *modifier of amylose extender 1* (*mae1*). The objectives of this research are to map the *mae1* mutation in a family of RILs segregating for *mae1* using GBS, to identify candidate genes for further analysis and to analyze the effect of *mae1* on kernel starch properties.

P283

Mapping of Quantitative Trait Loci for Salicylic Acid-induced Cell Death in intermated B73 x Mo17 (IBM) population

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Cell death plays a critical role in plant immune system. Plants use the hypersensitive response (HR), which is a strong and unique form of cell death, to restrict the spread of infection by biotrophic phytopathogens. The molecular pathway that controls HR and other forms of cell death in plants has yet to be elucidated. Salicylic acid (SA), a phytohormone highly accumulated during pathogen-triggered HR, induces cell death in maize plants and the strength of this SA-induced cell death is genetic-background dependent. To identify the quantitative trait locus (QTL) for SA-induced cell death in maize, we performed a QTL mapping with the intermated B73 X Mo17 (IBM) population which is a population derived from a cross between the maize lines B73 (low sensitivity to SA) and Mo17 (high sensitivity to SA). The QTLs identified from this mapping will be compared with the previously identified HR-associated QTLs to identify HR-specific QTLs.

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

P284

Marker selection for fingerprint and quality control for CIMMYT maize lines

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With the SNP (Single nucleotide polymorphism) detected method development, using SNP marker to do the germplasm fingerprint and quality control (QC) is becoming a very important component for germplasm maintain in genebank and breeding program. But how to select best SNP and how many SNPs used for the fingerprint and quality control is an important issue since it relates to the cost of single sample. The CIMMYT Maize Lines (CMLs) are a set of 561 inbred lines, developed over the past 60 years by CIMMYT breeders throughout the world. Develop a fingerprint and QC platform to help maintain this important germplasm has become an important issue to be solved in CIMMYT genebank. In this study, GbS genotype data of whole set of CMLs was used to develop a strategy for select best SNP for fingerprint and quality control. Finally, a subset SNP which include 80 best markers was identified for fingerprint, and 10 SNP was identified for quality controls with 99% identify power. And then both fingerprint and QC subset SNPs were verified using different re-generation samples. Our result give an idea how to select best SNP for fingerprint and quality control and also make a foundation for stablish a platform for CIMMYT maize inbred lines fingerprint and quality control.

Funding acknowledgement: SAGARPA

P285

Meta-QTL analysis for yield traits in maize

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Yield is one of the most important and genetically complex trait. Many studies have so far been carried out with the objective to map quantitative trait loci (QTLs) related with yield. These studies produced a large amount of QTL information which has been deposited in different databases and hundreds of scientific publications (Salvi and Tuberosa, 2015, The crop QTLome comes of age. Curr Opin Biotech). QTL meta-analysis enables to summarize QTL information, to identify consensus QTLs across studies and to refine QTL positions on a consensus map. Objectives of our study were to summarize the information about yield and related traits QTLs in maize by means of QTL meta-analysis and to highlight emerging properties related to QTL distribution along chromosomes. Information was collected from 48 published studies (from 1992 to 2014), including 29 different crosses produced from 39 inbred lines, and tested in 79 experiments. RIL was the most common population type (11 out of 29); genotypes ranged from 99 to 500; on average, 2.5 yield-related traits (such as grain yield per Ha, ear weight, ear number, etc) were considered per study. From this search, a total of 1,444 QTLs were initially collected, which reduced to 803, after filtering for obvious overlaps (e.g. same QTLs in different experiments of the same study). A total of 228 meta-QTLs were mapped, including 77 meta-QTLs resulting from multiple QTLs. QTLs projections on genetic or physical maps showed strikingly different distributions, with clear QTL clustering at centromeric regions on genetic maps.

P286

Metabolite-QTL analysis of surface lipid production on maize silks: building statistical frameworks for inferences of biochemical function

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During the critical period that maize silks are exposed to the environment for pollen reception, the silk's cuticle provides a primary line of defense against abiotic and biotic stresses (e.g., UV radiation, insect damage, desiccation). Silk surface lipids in maize primarily include fatty acids, aldehydes and hydrocarbons ranging in length from 16 to 35 carbon atoms, and are thought to play central roles in mitigation of stresses. However the genetic and metabolic networks responsible for production of these surface lipids remain undefined.

To understand how the silk cuticle fulfills its diverse functions, we have implemented a systems biology approach to comprehensively determine the enzymes, regulators and metabolic reactions involved in biosynthesis of the silk surface lipid metabolome. As an initial step, we performed metabolite-QTL (mQTL) mapping using 254 Intermated B73xMo17 recombinant inbred lines, which possess considerable variation in the surface lipid metabolome. QTL analysis of 65 constituent traits, 18 metabolite-class traits, and 13 relative composition traits identified a total of 350 mQTLs that modulate the abundance and composition of the silk surface lipid metabolome, specifically hydrocarbons. We detected genetic regulators for the accumulation of individual metabolites as well as for classes of metabolites (e.g. class = all alkanes of odd-numbered chain length). Using non-parametric confidence intervals (NPCIs), we demonstrate that positional resolution is very high in this experiment, with some QTL regions harboring only tens of genes. NPCI co-localizations of multiple mQTLs to narrowly defined positions suggest pleiotropic actions by the causal polymorphisms. To address the impossibility of directly testing null hypotheses of non-pleiotropy, we explore the statistical framework of equivalence testing for treating hypotheses that bear on functional inference. We will discuss how these cases are shaping our candidate gene lists and our models of surface lipid biosynthesis and regulation.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy Ames Laboratory, Iowa State University's Presidential Scholars Program

P287

Mild Inbreeding Depression in a Unique Synthetic Population: Preliminary Findings

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In an effort to identify and study rare and potentially deleterious alleles in maize and its progenitor (teosinte; *Zea mays* ssp. *parviglumis*), we are comparing paired inbred (S1) and outbred (S0) families derived from the Zea Synthetic population. The ZeaSyn population was created by random mating the NAM parents and 11 B73 x *parviglumis* BC_1 populations. A total of 924 ZeaSyn6 males were selfed and used to pollinate a random ZeaSyn female, creating the paired S1 and S0 families, respectively. Here we present preliminary year-one results from a two-year trial where we collected agronomic and fitness related traits in MO, NY, and NC. As expected, S1 families had lower agronomic and fitness values for the traits measured than S0 families. We have genotyped the parents of the S1/S0 families using genotyping-by-sequencing (GBS). To aid in the genotype imputation and allow for haplotype analysis, we have whole genome sequenced (WGS) the ZeaSyn founders. The genetic and phenotypic data will be combined in association mapping to identify rare and deleterious alleles affecting these traits.

Funding acknowledgement: National Science Foundation (NSF)

P288

Molecular characterization of doubled haploid exotic introgression lines in maize

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Increasing genetic variation is one of the ways to increase genetic gain. Exotic maize landraces from the Germplasm Enhancement of Maize (GEM) project were introgressed into the background of two inbred lines with expired plant variety protection (PHB47, PHZ51) through a single backcross generation, wherein doubled haploid (DH) lines were developed. The objectives of this study are to identify the percentage of donor parent introgression, and the specific regions where these introgressions are located in the genome of each GEM-DH line. A total of 230 GEM-DH lines were evaluated using 8,356 SNP markers. Initial results showed that, on the average, 11.1% of the markers are of exotic parent alleles. The percentage of donor parent introgression may be underestimated due to the inability to distinguish markers that are monomorphic between exotic and elite parents. Measures to correct for these monomorphic markers will be discussed. This study ultimately aims to identify donor parent introgression that could be sources of novel alleles of traits with economic importance.

Funding acknowledgement: United States Department of Agriculture (USDA), RF Baker Center for Plant Breeding, Department of Agronomy, Iowa State University

P289

Molecular mapping of gibberella ear rot resistance and kernel drydown rate in maize

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Gibberella ear rot resistance and fast kernel dry down rate are two important traits to improve the productivity of maize in short season areas of the temperate zone. To study the relationship between these two important traits and optimize breeding efforts, we evaluated 410 recombinant inbred lines (RILs), derived from the inbreds B73 and CO441, for GER resistance using artificially inoculated fields in one location for three years. These RILs were genotyped using genotyping-by-sequencing. In a separate experiment, we also evaluated the same set of lines for kernel drydown rate. Heritability estimates were moderately high (H = 0.76 – 0.80) for disease severity and kernel drydown rate. We observed significant (P < 0.01) but weak phenotypic (rp = 0.16) and genotypic (rg = 0.20) correlation between the two traits. Chromosomal regions associated with GER resistance that explained up to 48 % of the phenotypic variation were identified on chromosome 1, 3, 8 and 9. For the rate of kernel drydown, 20 % of the phenotypic variation was explained by regions on chromosomes 1, 6 and 8. One common region was detected on chromosome 1 for both traits with more impact on GER resistance than on kernel drydown. We recommend the use of this region in future marker assisted breeding efforts to improve both traits simultaneously.

Funding acknowledgement: Canadian Field Crop Research Alliance

P290

Optimizing tissue culture parameters for callus induction and regeneration of transgenic sorghum Lines.

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Sorghum [Sorghum bicolor (L.) Moench] is a C4 grass of African origin. It belongs to Poaceae family and is widely cultivated in diverse climates around the world for food and forage. Sorghum is one of the most important cereals in the world after rice, maize, wheat and barley. Its high genetic diversity can be utilized to improve economically important traits to meet the challenge of climate change and ever increasing food demands across globe. It has significant genetic homology with sugarcane and maize and is an attractive candidate for energy crop due to its high biomass, yield and sugar content. It is highly stress tolerant and has high water use efficiency due to its deep root system and reduced transpiration rate thus can grow on marginal soil with low nutrient and water inputs.

Candidate genes related to economically important traits like height, biomass, maturity, tiller number, kernel weight, saccharification has been mapped by QTL mapping and association mapping using SNP and SSR markers. Mini-core collection developed at International crop research institute for semi arid tropics were used for the mapping purpose.

The functional validation of the genes remains a challenge due to recalcitrant nature of sorghum genetic transformation. However, it is achievable by both biolistic (Casas et al. 1993) and Agrobacterium (Zhao et al. 2000) methods. We are using Agrobacterium mediated transformation protocols to optimize our tissue culture media for callus formation and regeneration of plants. Several explants like immature embryo, seeds, apical meristem, and leaf were used. several genotypes are tested along with different media and hormone concentration. MS based media with 2 mg per L 2,4-D and 0.2 mg per L kinetin have shown better results in callus formation. However it is too early to confirm the right media and genotype combination as several are on test.

Funding acknowledgement: United States Department of Agriculture (USDA)

P291

Pedigree-based approaches to identifying selection in US maize

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Modern maize has resulted from generations of mass selection for fitness (yield, standability, maturity, pest resistance, etc). Yet, in terms of adaptation, data from genome scans have largely fallen short in identifying anything except hard selective sweeps in modern maize. This shortcoming may be due to the failure to account for population structure in the data. Here we explore whether pedigree data allows identification of weak selection and selection on standing variation. We used reduced-representation phased GBS data from more than 2500 maize recombinant inbred lines representing over 60 years of US maize germplasm. We reconstructed pedigree graphs using identity by descent (IBD) or identity-by-state (IBS) information and compared these to known historical parentage records for robustness. Finally, we evaulated the power of our pedigree approach to identify loci targeted by selection during modern maize breeding.

Funding acknowledgement: United States Department of Agriculture (USDA), Dupont Pioneer

P292

Phenotypic Characterization of Traits Related to Perenniality in Maize/Teosinte

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During the summer of 2014, traits related to perenniality were characterized in maize/teosinte hybrids in a field plot at the University of Georgia. The observed germplasm was generated by first crossing maize B73 with its closest diploid perennial relative, *Zea diploperennis*, during the winter of 2012. Eight seeds were generated from the cross and planted outside for the summer of 2013. The eight plants were sib-crossed and generated approximately 2,000 seeds. From this germplasm, 485 F2 hybrid plants were grown in a field plot and characterized for traits related to perenniality over the course of the summer of 2014. Plants were characterized based on the following traits: tassel emergence date, pollen shed date, silk emergence date, kernel row number per ear, number of tillers, and the presence or absence of regrowth after flowering. Trait frequency distributions in comparison to parental phenotypes is currently being done as well as generating GBS markers for future QTL mapping.

P293

Phenotypic evaluation of doubled haploids derived from the Zea Synthetic population

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The biology of rare alleles (<5% frequency) is fundamental to our understanding of evolution and genotype-to-phenotype relationships, and has yet to be adequately explored in any system. Here, we investigate the contributions of allelic variants throughout the maize genome to fitness for both teosinte (*Zea mays* ssp. *parviglumis*) and maize (*Zea mays* ssp. *mays*) in a common population, the Zea Synthetic. The Zea Synthetic was created by crossing two other synthetic populations: the Nested Association Mapping (NAM) Synthetic (created from the NAM founders) and the Teosinte Synthetic (created from BC1s between B73 and 11 geographically diverse *parviglumis* accessions). The Zea Synthetic was random mated for four generations prior to the start of this project. The expected parentage of the Zea Synthetic is 38% B73, 2% each NAM parent, and 12% teosinte. For this study, AgReliant Genetics has created 2000 doubled haploid (DH) lines from the Syn-4 generation. To date we have evaluated the DH for a number of agronomic (flowering, PH/EH, number of ears) and fitness (total number of progeny and progeny weight) traits at four locations. Genotyping-by-sequencing (GBS) data on the DH will be used to determine identity by descent from the original parents for each region of the genome. Comparison of allele frequencies in the outbred generation to those of the DH lines will indicate which regions of the genome underwent selection during the DH process. Furthermore, we will determine the origin of alleles that may have been purged, whether from a NAM founder or from teosinte, to address the hypothesis that purging will be stronger on teosinte alleles. Genotypic and phenotypic data will be combined in an association analysis to determine phenotype and genotype relationships.

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

P294

Pooled GBS: Cost-effective and background independent genetic mapping of mutants and QTL

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Genetic mapping of new mutants, which allows us to map a mutant phenotype to a causal locus or loci in the genome, is a crucial step in forward genetics. Construction of a mapping population that consists of mutant and normal individuals is essential for genetic mapping. The mapping population can be used by different high-throughput methods for genetic mapping. Single Nucleotide Polymorphism (SNP) arrays and Sequenome-based methods detect presence and absence of pre-discovered SNPs, and therefore are not background independent. In contrast, high-throughput sequencing (HTS) based methods used for genetic mapping are generally background independent. Some HTS methods such as Genotyping-by-sequencing (GBS) and Rapid-seq use DNA for mapping, while other methods such as BSR-seq and MMAPPR use RNA. Current DNA-based methods barcode DNA extracted from each individual in the mapping population to construct the sequencing library, and RNA-based methods construct a separate library from each of two pools, namely mutant and normal. Both approaches provide high resolution maps to identify causal loci, but are not cost-effective for screening a large number of mutant families such as may be recovered from an enhancer/suppressor screen. Here we present a low-resolution, but cost-effective, HTS-based method for genetic mapping. We pool tissue from phenotyped individuals to create a mutant and a normal pool, and adapt the original GBS method to construct sequencing libraries. We have applied this method to map mutants resulting from an enhancer/suppressor screen. Our method is cheaper than the current GBS protocol, easier than using RNA for library construction, and without the biases inherent in sampling polymorphisms from the RNA expressed in a certain tissue type(s). Here we present the pipeline and results from these genetic mapping efforts.

Funding acknowledgement: National Science Foundation (NSF)

P295

Predicting biomass yield in photoperiod-sensitive sorghum

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Phenotypic prediction using genotypic information and a trained model can accelerate genetic gain in plant breeding. We investigated the use of genomic selection (GS) and genome-wide association (GWAS) to predict biomass yield and its component traits (plant height, lodging, and moisture) in biomass sorghum, a new bioenergy crop. A panel of 415 photoperiod-sensitive sorghum accessions was evaluated over three years (2012-2014) for plant height, lodging, moisture, and total biomass yield. Heritabilities ranged from 0.39 for biomass yield to 0.85 for plant height. Three GS models (GBLUP, LASSO, and Elastic-net) and three GWAS models (CMLM-P3D, MLM-P3D, and MLM_noP3D) were compared for each trait. Since the 2012 trial used smaller plots and had lower heritabilities, we assessed whether inclusion of the 2012 data was helpful for GS and/or GWAS. The GBLUP model had the highest prediction accuracy for all traits, and models were improved by including 2012 data. Significant associations were detected ($q<0.1$) for plant height, lodging, and moisture, but not biomass yield. We are investigating the use of phenotypic data and trait associations for higher-heritability component traits (plant height, lodging, and moisture) to improve prediction of a lower-heritability target trait (biomass yield).

Funding acknowledgement: Department of Energy (DOE), Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES)

P296

Prediction accuracy of QTL models improved by ensemble models

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Within linkage mapping populations, linkage disequilibrium due to finite population size and tight linkage between dense markers can cause collinearity among marker genotypes, complicating detection of QTL and estimation of QTL effects. Thus, very high density linkage maps do not provide better QTL models than moderately dense maps. Here we developed an ensemble approach to QTL mapping to enable the full use of high resolution linkage maps while avoiding collinearity problems that would otherwise occur with high density linkage maps. The ensemble approach maintained aspects of marker selection that characterize standard QTL mapping but incorporated information from more relevant markers. The objective of the new method was to improve prediction power compared to QTL mapping while provide more specific insights into genetic architecture than genome-wide prediction models. Results demonstrated that the proposed ensemble approach can substantially improve prediction ability for both biparental and multi-family mapping populations in QTL-based genomic prediction. Moreover, the true genetic architectures of the complex traits may be somewhere between a few major QTL controlling the trait and a pure polygenic inheritance, so an ensemble model combining QTL-based models and the infinitesimal GBLUP model might offer further improvement in the accuracy of genomic predictions. We compared the prediction accuracy of the ensemble of joint linkage model and the GBLUP model to the accuracy of the component models.

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P297

QTL mapping of resistance to Fusarium ear rot and fumonisin contamination in four NAM families

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Fusarium verticillioides causes Fusarium ear rot (FER) and produces the mycotoxin fumonisin in maize, which can result in devastating yield losses and serious human health risks, respectively. Resistances to FER and fumonisin contamination are quantitative, and previous studies have suggested that the two traits are moderately to highly heritable and strongly correlated with each other. Here, quantitative trait loci (QTL) for resistance to FER and fumonisin were mapped in four families of recombinant inbred lines (RILs) from the maize nested association mapping population. The four families (CML52xB73, CML69xB73, CML333xB73, NC358xB73 RILs) were grown and inoculated with *F. verticillioides* in Clayton, NC from 2012-2014. FER was scored after all three seasons, and fumonisin content was quantified in 2013 and 2014. Single family QTL analyses included standard interval mapping and step-wise regression, and the four families were combined for joint step-wise regression. In this material, FER and fumonisin contamination were not correlated and their associated QTL did not colocalize. However, further analyses revealed family-specific relationships between the two traits.

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P298

Rapid Hemispherical Photographic Phenotyping of Productivity and Canopy Dynamics in a Setaria RIL Population

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Crop genetics and breeding is limited by the ability to accurately gather useful phenotypic information from large, diverse populations of crop genotypes. Most significantly, rapidly and non-destructively assessing the productivity and allometry of crop plants at high frequency during a growing season in the field remains a significant challenge. Hemispherical photography has proven utility in forestry research as a tool to evaluate the growth, structure, and light interception of a canopy. But, high resolution digital cameras with fish-eye lens have been large and relatively expensive. This study tested the use of a small digital camera (GoPro Hero3+) customized with a fully hemispherical lens and miniature self-leveling gimball to rapidly assess leaf area index, biomass production and radiation interception efficiency for quantitative trait loci (QTL) analysis in a diverse population of 186 recombinant inbred lines (RILs) generated by crossing Setaria viridis with S. italica. Plant area index estimated from hemispherical photographs correlated strongly with leaf area index ($r^2 = 0.85$), stem biomass ($r^2 = 0.76$) and total vegetative biomass ($r^2 = 0.84$) in an initial validation experiment where LAI varied over time and genotypes from 0.3 – 3.6. Using these trait regressions, canopy hemispherical photography was then analyzed from the full population of RILs. QTL analysis will be presented comparing loci identified from conventional biomass harvests and the rapid hemispherical photographic phenotyping.

Funding acknowledgement: Department of Energy (DOE)

P299

Recovering Power in Association Mapping Panels with Variable Levels of Linkage Disequilibrium

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Association mapping has permitted the discovery of major QTL in many species. It can be applied to existing populations and, as a consequence, it is generally necessary to take into account structure and relatedness among individuals in the statistical model to control false positives. We analytically studied power in association studies by computing noncentrality parameter of the tests and its relationship with parameters characterizing diversity (genetic differentiation between groups and allele frequencies) and kinship between individuals. Investigation of three different maize diversity panels genotyped with the 50k SNPs array highlighted contrasted average power among panels and revealed gaps of power of classical mixed models in regions with high linkage disequilibrium (LD). These gaps could be related to the fact that markers are used for both testing association and estimating relatedness. We thus considered two alternative approaches to estimating the kinship matrix to recover power in regions of high LD. In the first one, we estimated the kinship with all the markers that are not located on the same chromosome than the tested SNP. In the second one, correlation between markers was taken into account to weight the contribution of each marker to the kinship. Simulations revealed that these two approaches were efficient to control false positives and were more powerful than classical models.

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P300

Reducing pre-harvest losses from aflatoxin in maize production through integrated breeding and pest management strategies: initiation of a five year project

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Aflatoxin is a highly carcinogenic and highly regulated mycotoxin produced by the fungus *Aspergillus flavus* in maize when plants are under stressful (unfavorable abiotic and biotic) conditions. Aflatoxin contamination of maize is a threat to food security in both the US and worldwide. It is a recurring problem in the Southern US causing substantial economic losses for growers and the problem will likely expand toward the Midwestern Corn Belt under a changing climate. We hypothesize that sufficient mitigation of aflatoxin requires integrated strategies of breeding for adaptation and known genetic resistances, appropriate management of insects and abiotic stresses, and the application of atoxigenic strains. In this first year we are in the process of:(1) developing improved germplasm and genetic lines to lower aflatoxin risk and enhance abiotic stress tolerance; (2) validating previously reported aflatoxin resistance QTLs in crosses with high yielding inbred lines; (3) characterizing lines for release with multiple stress resistance, focusing on aflatoxin reduction and resistance to other associated factors (e.g., drought tolerance, insect resistance) for commercial applications; (4) optimizing innovative and integrated management approaches to reduce aflatoxin, characterizing contributions from crop genetics, crop stress and pest management, atoxigenic strains and their interactions; (5) educating students and producers on a comprehensive, integrated aflatoxin management strategy, through extension meetings and multi-media publications, and directed partly from research conducted on grower co-operator farms. By integrating this project with farmers and other stakeholders across research, extension and education, new approaches for encouraging loss reduction will be identified.

Funding acknowledgement: United States Department of Agriculture (USDA)

P301

Reproductive Resilience of Drought Tolerant Hybrids under Water-Limited Conditions.

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Drought is the most common abiotic stress constraining productivity of rain-fed crops. In 2011, the DuPont Pioneer breeding program successfully developed drought tolerant maize hybrids, launched as Optimum® AQUAmax™ product. Drought stress during flowering has the most deleterious impact on maize yield, interrupting the kernel set process and reducing yield potential. We used a field pot phenotyping approach to precisely impose drought stress at flowering to Optimum® AQUAmax™ and drought susceptible hybrids to identify key phenotypic differences in reproductive resiliency at this critical stage. Vegetative and reproductive traits were collected under well-watered (WW) and flowering stress (FS) conditions. FS reduced plant growth rate and plant height but had little or no effect on the leaf appearance rate. However, FS had a significant effect on reproductive traits which specifically discriminate between Optimum® AQUAmax™ and drought susceptible hybrids. For example, under FS conditions, Optimum® AQUAmax™ hybrids demonstrated more aggressive silking and a shorter ASI (anthesis to silking interval) compared to drought susceptible hybrids. However, total initiated spikelet number per ear was not affected by FS in either Optimum® AQUAmax™ or drought susceptible hybrids. Tip spikelet arrested development and/or post-pollination abortion was increased in FS, compared to WW but tended to be less impacted in the Optimum® AQUAmax™ hybrids. This resulted in more robust post-pollination ear growth in the Optimum® AQUAmax™ hybrids under FS conditions. These findings suggest that genetic programs controlling pre-pollination organ initiation and differentiation are less sensitive to environmental stresses compared to post-pollination organ growth and expansion.

Funding acknowledgement: Dupont Pioneer

P302

Results of recurrent selection for large endosperm size in a supersweet population

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Sweet corn with the *shrunken-2* mutation has higher sugar content, longer shelf life, and is generally preferred by consumers over the *sugary1* mutant. However, *sh2* has poorer germination and seedling vigor relative to *su1*. The kernels have reduced carbohydrates, and therefore reduced energy for the growing seedling. Since embryos are rich in oil, one way to increase energy resources would be to select for larger embryos. Seven generations of recurrent selection were carried out in a population created by crossing the *sh2* hybrid Challenger by an Illinois High Oil derived line, with the goal of increasing embryo size. Each cycle was selected based on the two-dimensional embryo area as viewed from the ventral face of the dry kernel. The direct target of selection, embryo area, was found to be responsive to selection and increased linearly over seven cycles. In total, embryo area increased 35.8 percent. Embryo weight also increased by 32.5 percent. Whole kernel and endosperm weight also increased by 22.1 and 15.1 percent, respectively. Germination was unresponsive to selection and not correlated with embryo or kernel size.

Other factors were examined to determine indirect effects of selection. It was found that selection had no effect on kernel composition. Selection had a negative effect on numerous agronomic characteristics: plant height decreased by 11.5 percent, ear height decreased by 13.5 percent, yield decreased by 7.8 percent, row count decreased by 10.4 percent, and growing degree days to flowering decreased 1.8 percent. Tassel morphology also changed over the cycles of selection with a 5.1 percent increase in abnormal tassels. Lastly, kernel color changed linearly over the cycles of selection: the number of ears homozygous for white endosperm increased by 50.8 percent and ears homozygous for yellow endosperm decreased by 29.0 percent.

Funding acknowledgement: National Institute of Food and Agriculture (NIFA)

P303

Rice Nested Association Mapping Population and its Phenotyping

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Nested association mapping (NAM) combines the major genetic methodologies, QTL mapping and association mapping. To examine the genetic architecture of rice complex traits and the allelic variation, two rice NAM populations have been developed: JNAM is derived from Koshihikari/Japanese rice diverse cultivars and WNAM is from Taichung 65/World rice diverse accessions. Koshihikari is the leading cultivar in Japan whereas Taichung 65 shows worldwide adaptable. Our phenotyping system using a large number of rice populations in our paddy field has been also revised. We are going to show and discuss our NAM and phenotyping system.

Funding acknowledgement: Grants-in-Aid for Scientific Research (A) MEXT No. 25252002, Council for Science Technology and Innovation Cross-Ministerial Strategic Innovation Promotion Program “Technologies for Creating Next-Generation Agriculture Forestry aand Fisheries”

P304

Samples with high carotenoid and anthocyan contents in maize heterosis breeding

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Significant progress in maize breeding provides the universality of its food and feed utilization. First of all it is connected with maize capability to plant and grain chemical alterations in any prescribed direction. One of such important directions is the enhancement of grain quality through the augmentation of vitamin maintenance, in particular, carotenoid complex. Among milk and poultry manufacturers specific interest is induced by new maize carotenoid feed products. Agricultural Steppe zone Institute of the National Academy of Agrarian Sciences of Ukraine has been developing the program on searching and selection of initial materials with corresponded grain abilities for food and feed maize since 2003. As a result the samples with carotenoid contents twice-three times higher than usual maize samples, with high anthocyan maintenance were discovered. Identified line DK772ZMCV with high carotenoid contents in grain has been registering in Ukraine since 2014 and is one of parental components in maize hybrids DN Bagrjanij, DN Rubin, Cherry, and hybrid RedWine which is transferred to state variety testing now. High carotenoid hybrids have been registered in Ukraine in general list “Maize habitual” and is not inferior to usual grain maize forms in productivity. Generated hybrids include only one carotenoid component with dominant type of inheritance, so the creation of valid heterosis model of a high carotenoid hybrid is the first-priority goal. For MAS on anthocyan contents the comparison of 230 inbreds with anthocyan ear cob and 68 inbreds with white ear cob on 384 SNP-markers has been carried out. The total amount of SNP-markers which ensured the significant disequilibrium of major allele frequencies between two inbred groups put together 56,9%. Ten top SNP-markers which had the most remarkable change of major allele frequencies in alternative groups were identified as SNP-markers of anthocyan coloration of ear cob.

Funding acknowledgement: National Academy of Agrarian Sciences of Ukraine

P305

Sink and Source Potential of Long-Ear Genetics

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Short-season maize hybrids are source limited, meaning their ability to accumulate dry matter is what limits grain yield. This research examines the potential of BSLE(M-L)C30 [i.e., Long Ear (LE) genetics] to enhance source capacity without compromising sink potential of short-season hybrids. Preliminary results from a study using hybrids between unselected inbred lines from BSLE(M-L)C30 and 2 short-season inbred lines (CG60 & CG102) are promising: (1) sink potential of LE hybrids is greater than a Conventional Ear (CE); (2) grain yield on a per plant basis of several LE hybrids is greater than the CE hybrid; (3) flowering time of LE hybrids is equivalent to CE hybrids; and (4) source potential appears to be greater in several LE hybrids than the CE hybrid.

Funding acknowledgement: Natural Sciences and Engineering Research Council of Canada (NSERC), Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), and Grain Farmers of Ontario (GFO)

P306

sRNAome-based prediction of yield heterosis in maize

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Heterosis describes the superior performance of hybrid offspring over its parents and is of high relevance to hybrid breeding and agriculture. Hybrid breeding programs requires intensive field trials for the selection of optimal inbred line crosses. Genomic selection based on genetic markers, SNPs, transcriptome data etc. allows for a preselection of most promising individuals for future crosses based on computational prediction and thereby increases the efficiency of the selection process.

Small, non-coding RNAs (sRNAs) are known to be involved in the regulation of gene expression and the modulation of the epigenomic landscape. sRNAs undergo tremendous expression changes in plant hybrids relative to their inbred parents. Additionally, they are attributed to play fundamental roles for the specification of hybrid phenotypes and thus are promising biomarkers.
We conducted a study to identify the degree of the relation of small RNA expression profiles from seedling material of elite maize inbred lines with yield heterosis in their hybrid offspring. Our analyses revealed an overall negative relation of differences in sRNA transcriptomes of inbred parents with yield heterosis in the hybrid offspring, opposing to mRNA-transcriptome or SNPs. This finding suggests an restraining effect of sRNAs on yield heterosis. We found significant associations for distinct sRNAs with yield heterosis. Genomic predictions based on associated sRNAs resulted in high prediction accuracy of the heterotic outcomes, superior to AFLP-, SNP- and mRNA transcriptome-based predictions.

The uncovered restraining effect of sRNAs on heterosis and the high prediction accuracy suggest that future plant breeding strategies that include sRNA expression profiles should enable to generate unprecedented levels of heterosis and considerably increase crop yields.

Funding acknowledgement: DFG

P307

Study of Quantitative Trait Polymorphisms Emerging From Doubled Haploids Maize Lines

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Double haploids are useful in plant breeding and genetics. Because they are expected to be completely homozygous, the progeny of these doubled haploids are expected to be genetically homogeneous and, except for rare mutations, should show no genetic diversity. Even so, over 50 years ago George Sprague and his associates demonstrated that heritable variation in quantitative traits quickly emerged among the progeny of doubled haploids. Sprague demonstrated that the rate of variation was greater than the rate of spontaneous mutations, but he did not have the means to determine the source of that variation. We believe that, with new technologies, the means now exist. We are in the process of establishing and identifying heritable polymorphic lines that have descended from a single doubled-haploid B73 plant. In the summer of 2014 we planted seed for two sequential generations from each of ten lineages. These were planted in triplicate in a randomized complete block design (RCBD). The resulting plants were evaluated for fourteen quantitative traits (plant height, number of tassel branches, 100 grains weight, etc.) and the results are currently being statistically analyzed. A heritable polymorphism for any particular trait is indicated if there is no significant difference between the two generations of a lineage but the lineage is significantly different from the other lineages. We will be presenting preliminary findings.

Funding acknowledgement: SDSU Agriculture Experiment Station

P308

The Genetic Architecture of Maize Photoperiod Sensitivity Revealed by Multiple-parent Populations

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Photoperiod sensitivity is one key component to modulate maize flowering time by day length. The reduced photoperiod sensitivity is required for maize flowering under long-day condition due to maize postdomestication adaptation from short-day condition. We measured maize flowering time of 10 independent RIL (recombination inbred line) populations (maize Random Open-parents Association Mapping population, ROAM) in the field under both short and long day lengths. A total of 557, 553 and 469 loci were identified for 20 traits related to maize flowering time under both conditions and photoperiod responses of flowering time in ROAM population by using separate linkage mapping (SLM), joint linkage mapping (JLM) and genome-wide association study (GWAS), respectively. Among these loci, 224 common loci were detected for the consistent trait by at least two models. Nearly half of the QTL (43.6%) for flowering time were overlapped between short- and long- day environments, which seemed to function through endogenous pathways or other environmental signals. About 15.2% loci were detected to be associated with photoperiod response, which influence maize flowering time through photoperiod pathways. These results may provide deep understanding of maize postdomestication adaption.

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P309

Uniting the world's popcorn diversity for the dissection of complex traits and accelerating breeding

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Popcorn is the only type of maize that pops. While nearly all of the world's popcorn production is in the US, the consumers of popcorn are worldwide. US popcorn growers have benefitted from over a century of selection on diverse germplasm that came to them from all over the world. Nearly all of the popcorn consumed in Mexico comes from the US, because US popcorn can pop 24-fold bigger than Mexican popcorn. With such a huge differential, landrace popcorn growers in Mexico are unable to compete for a market share, so their number decreases every year. In the CIMMYT Maize Germplasm Bank, we have 873 popcorn accessions with ample phenotypic variance. They provide a valuable resource to find the best genetic diversity for popping traits, and to determine the genetic basis for these traits. We have phenotyped 873 landrace accessions plus nine commercial checks for seven traits, including time to first pop, expansion volume, color of endosperm, shape of flake, pericarp remaining after popping, number of unpopped kernels, and weight of unpopped kernels. For time to first pop, 157 accessions pop faster than the nine commercial checks. For expansion volume, 11 accessions expand to a similar size or larger than commercial checks. Over a quarter of the kernels did not pop in the commercial popcorns, while 39 landrace accessions can pop with less than 5% of the kernels remaining unpopped. In conclusion, for every commercially important trait we measured, we identified landrace accessions that were equivalent or even exceeded the commercial checks. We will develop inbred lines to construct an association mapping panel and bi-parental populations to study the genetic basis of popcorn traits. Our goal is to develop the best popcorn varieties that are locally adapted to the agroecosytems of the countries targeted by CIMMYT's mission, starting with Mexico.

Funding acknowledgement: CGIAR Research Program for Managing and Sustaining Crop Collections

P310

Utilizing Evolutionary Conservation Information to Improve Prediction Accuracy in Genomic Selection

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Genomic selection (GS) has gained popularity recently as the availability of genome-wide markers has increased. Current methods for GS weigh all the available SNPs equally in model training, without considering their functional differences. Genetic variations detected at evolutionary conserved sites tend to be deleterious and, thus, may be more informative for GS. To utilize this kind of information as a prior into the GS model, we proposed a method to put more weight on evolutionarily constrained sites. As a proof-of-concept, a half diallel population based on 12 diverse inbred lines was used, from which seven phenotypic traits were collected. Some of these traits show high levels of heterosis. After sequencing the 12 founder lines, about 14 million SNPs were discovered and the SNPs were used to identify 502,913 haplotype blocks shared through identity by descent (IBD). A five fold cross-validation experiment was conducted using the summary statistics of the SNP conservation scores, which were computed by evaluating sequences similarity of multiple divergent species, in the IBD blocks as explanatory variables. The results show that the prediction accuracies are significantly better than shuffled data with randomly assigned conservation scores. This study demonstrates the importance of incorporating evolutionary information in GS and its potential use in plant breeding.

P311

Variation in yield loss to ozone of diverse inbred and hybrid maize lines

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Tropospheric ozone is an air pollutant that costs ~$14-26 billion in global crop losses and is projected to worsen in the future. Fifty-two inbred lines, including the nested association mapping (NAM) population founder lines, and 26 hybrids made from crossing B73 to the NAM founders were tested for ozone sensitivity under ambient (40 ppb) versus elevated ozone concentrations (100 ppb) at the Free Air Concentration Enrichment (FACE) site in Illinois in 2014. Across all inbred genotypes, total ear mass was 7% lower at elevated ozone. However, there was significant genetic variation in response with yield loss ranging from 0% in tolerant genotypes to 76% in the most sensitive genotype. Likewise, yield across hybrid genotypes averaged 9%, but varied from 0 – 26%. Notably, maize reference line B73 was insensitive to growth at elevated ozone whereas Mo17 showed significantly lower ear mass, delayed silking and lower ear height. In addition, yield loss of 17% was observed in hybrid B73 x Mo17. These results suggest that the extensive germplasm resources available for quantitative genetic analysis of phenotypic variation in B73 and Mo17 will facilitate investigation of oxidative stress tolerance in maize. Ultimately, the variation in yield loss to elevated ozone among genotypes has the potential to be exploited to improve the stress tolerance of maize. Future work includes completing the analysis of yield components, the assessment of key vegetative and reproductive traits and to determine the transcriptional responses and genetic loci associated with ozone sensitivity.

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P312

Yield and hybrid vigor within hybrids from inbreds preserved at the USA maize collection

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Recent advances in Next Generation Sequencing (NGS) have provided us with new tools to explore the maize genome and the genetic architecture underlying important quantitative traits. Using NGS information, progress has been made in understanding how some developmental traits are regulated (e.g., flowering time, plant height). However, challenges remain; very little is known about how these traits or more complex traits (i.e., yield) are genetically controlled in maize hybrids. The main objective of our study is to expand our understanding of the genetic architecture underlying quantitative trait loci variation in maize hybrids. We evaluated a set of hybrids created by crossing a subset of the US National Maize Inbred Collection - those whose flowering-time ranges were similar to B73 – with a non-stiff stalk and/or stiff-stalk expired PVP line (PHZ51 and/or PHB47). More than 1,100 hybrids were evaluated, in 10 environments across the US over two years, for a range of developmental traits, as well as yield. The inbred lines used were genotyped using GBS (Genotyping by Sequencing). Finally, we imputed HapMap v3.1 data (a reference higher density SNP set) onto the GBS results. We looked at how additive and dominant effects are distributed across the maize genome for different traits and how that distribution is related with other parameters obtained from genomic data, like recombination or Fst between heterotic groups. In this poster we will present preliminary findings from this study.

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

P313

Zbrowse: An interactive GWAS results browser

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We have developed an interactive GWAS results viewer that is an extension of the classic GWAS Manhattan Plot. Zbrowse runs on a personal computer, but is displayed in a web browser and allows for the rapid graphical comparison of GWAS experiments performed on complex traits such as multiple phenotypes measured in multiple locations. The manhattan plots are fully interactive. The browser allows zooming by dragging. Clicking a point in a genome or chromosome-wide view quickly zooms in close enough to see genes under the point. Results can be filtered to only display overlapping QTL between experiments. In addition, results with base pair ranges, such as joint linkage support intervals, can be viewed on the same plot as the GWAS results to quickly visualize overlaps. The browser allows for easy and interactive navigation between plots displaying the entire genome, down to a plot less than a mega base wide displaying gene tracks. Genes under peaks can be clicked to open a browser tab with more information about the gene and all genes under a peak can be viewed in table form in the browser or exported as a comma-separated table. We use Maize NAM data from ionomics experiments to demonstrate the function of the browser.

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

P314

A sequence-indexed single gene knock-out resource for maize

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A sequence-indexed, user-friendly, reverse genetics resource is highly desirable 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 to: (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, enables 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 and made available at the Maize Genetics Stock Center for distribution. (2) More than 40,000 C' revertants bearing a *trDs** have been selected from lines with a high reversion frequency. Over 90% are heritable, showing that the system is extremely efficient for recovering germinal *Ds** transpositions. (3) By NGS of 3-D pools, more than 3,500 *trDs** target sites have been mapped to the reference genome with our publicly available pipeline InsertionMapper and others are presently being mapped. (4) Sequence annotation shows that more than 2,000 insertions are in genes. (5) All the lines generated in this project are listed in our web-searchable database, and, more than 2,300 of them have already been sent to the Maize Genetics Stock Center for distribution.

Funding acknowledgement: National Science Foundation (NSF)

P315

Analysis of small RNA expression in maize normal and segmental duplication stocks

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Small RNAs (sRNA) are relatively short (<100 nucleotides) segments of RNA which can be important regulators in plant development. MicroRNAs (miRNAs; 20~22 nucleotides long) are the best-characterized class of plant small RNA. They can down regulate their target genes and thereby affect many aspects of plant development. The identification and analysis of small RNAs and their target genes is currently of great interest. Many small RNAs in maize have been identified and analyzed by traditional methods and bioinformatic approaches, but in only a few cases have the functions of small RNAs been defined. In addition, little is known about the effects of segmental duplications on the expression of small RNA. Maize is one of the most widely cultivated and consumed plants around the world, and it is also accepted as a model plant in genetics research. Segmental Duplications (SDs) play an important role in maize genome variation by creating new genes and remodeling genome structure. In recent work we have isolated a series of inverted and direct SDs generated by Alternative Transposition of maize *Ac/fAc* transposable elements (Zhang et al. 2013; Zhang et al., 2014). These SDs range in size from several kb to > 10 Mb; one SD of 14.7 Mb exhibits strong dosage-dependent effects on phenotype, mRNA and small RNA levels. We will present our latest results regarding the expression of maize small RNAs, including a comparison of their expression in maize normal and segmental duplication stocks.

Zhang J, Zuo T, Peterson T. 2013. Generation of Tandem Direct Duplications by Reversed-Ends Transposition of Maize Ac Elements. PLoS Genet 9(8): e1003691. doi:10.1371/journal.pgen.1003691

Zhang, J., Zuo, T., Wang, D., and Peterson, T. 2014. Transposition-mediated DNA re-replication in maize. eLife 2014;3:e03724

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P316

Characterization of DNA methylation level in the *Tourist* transposable element and flanking region in the control region of maize domestication gene *tb1*

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During domestication, maize underwent dramatic transformation in both plant and inflorescence architecture from its wild progenitor, teosinte. Mapping of a domestication QTL contributing to increase in apical dominance led to the discovery of *teosinte branched1*(*tb1*) gene. Further mapping and population genetics analysis indicated that the region controlling phenotype differences between maize and teosinte lied between −58.7 and −65.6 kb of the *tb1* ORF. Within this control region, there were two components capable of regulating *tb1* independently, namely the proximal and distal components. Transient expression assays showed that the *Hopscotch* retrotransposon in the proximal component of maize acted as an enhancer, which was consistent with the known higher *tb1* expression level in maize compared to teosinte. However, the candidate polymorphism in the distal component, a *Tourist* transposon (MITE) in maize, failed to induce differential expression. My hypothesis is that the flanking region of the MITE has higher methylation level spreading from the MITE in maize compared to corresponding region in teosinte and that this methylation affects gene expression.Cytosine methylation is known to be associated with transposons and influence gene expression, which leads to my investigation on DNA methylation patterns in the maize and teosinte distal component. I assayed the methylation state at transposons (*Tourist* and *Hopscotc*h) and their flanking region using seedling leaf and immature ear samples from introgression lines with different proximal and distal components through McrBC-PCR and Bisulfite sequencing methods. F2 population of those introgression lines were also phenotyped for traits that are known to be regulated by *tb1*. However, I did not find any significant difference in methylation level between flanking regions in maize and corresponding region in teosinte. The factor affecting phenotype in the distal component may lie in the unselected region, which is not included in the luciferase construct and methylation detected region.

Funding acknowledgement: National Science Foundation (NSF)

P317

Characterization of full-length candidate genes capturing by Helitrons in B73

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Helitrons are a type of transposable element that have been reported in numerous species of plants and animals. *Helitrons* are unique from other types of transposable elements because they have the capability to capture gene fragments and carry them throughout the genome. However, they are difficult to identify because they lack the classic repeat characteristics typically found in other types of transposable elements. Many characteristics of *Helitrons* are still unknown due to their relatively recent discovery and the difficulty of locating and identifying them within genomes. It has been documented that *Helitrons* can capture pseudo-genes and partial gene fragments within their structures, and these fragments can be highly varied. We have been annotating sequences that have been verified as *Helitrons* by the Institute for System Biology's HelitronScanner software in the maize B73 inbred line. We first used the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST) to search the *Helitrons* sequences for potential full-length gene captures. Once these potential full genes in *Helitrons* have been identified, they will be analyzed to see if these genes have expressed sequence tag (EST) support and other functional protein domains. Our preliminary results show that a few *Helitrons* contain promising full-length candidate genes in B73 genome.

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P318

Characterizing and mapping of *Transgene reactivated 9*

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We have applied a forward genetic screen using ethyl methanesulphonate (EMS) mutagenesis to obtain 10 mutations in genes which are required for transcriptional gene silencing and some examples of RNA-directed DNA methylation. These mutants are collectively known as *transgene reactivated (tgr)* mutants, including *tgr9*. Whole genome methylation analysis indicates that *tgr9* phenotype may be distinct from *mop1* phenotypes, indicating *tgr9* may have a more locus-specific effect on methylation. B-A translocations were used to map the *Tgr9* gene to a specific chromosome and genetic mapping by IDP markers confirmed the result. In order to further locate *Tgr9*, we will take advantage of next generation sequencing (NGS) combined with bulk segregant analysis. This method has been successfully used in maize as well as other species. Identification of this gene will help us understand the diversity of transcriptional silencing pathways in maize.

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P319

Differential DNA methylation of 19-kDa zein genes in maize

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DNA methylation, prevalent in plant genomes in both symmetric and asymmetric sequence context, plays an important role in regulating gene expression. In maize, only a single zein gene is highly expressed in each of 19-kDa gene clusters (A and B types), z1A2-1 and z1B4. Taking advantage of somatic cell differentiation, we investigated the DNA methylation of individual zein gene copies in both promoter and gene body regions of leaf, normal endosperm, and cultured endosperm. Indeed, expressed genes have much lower methylation levels in promoter regions than silent ones in both leaf (non-expressing tissue) and normal endosperm (expressing tissue). Tissue-cultured endosperm, however, resets the DNA methylation pattern and tissue-specific gene expression. Gene expression was suppressed by increased DNA methylation and activated by reducing DNA methylation of z1B genes, but not of z1A gene copies. Furthermore, DNA methylation of the gene body was higher in leaf than in endosperm, whereas no significant difference was observed in gene bodies between expressed and non-expressed gene copies, whereas median CHG methylation (30%) might explain highly expressed compared to lowly expressed genes. These results reveal that differential methylation of zein gene copies is susceptible to tissue culture and influences gene expression. Because tissue culture is used to produce transgenic plants, these studies provide new insights into variation of gene expression of integrated sequences.

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P320

Distinct mechanisms of chromatin related silencing pathways of the *ufo1* and *Mop1* genes in maize.

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The small interfering RNA (siRNA) pathway has been widely researched in maize as it plays an integral role in gene silencing of transposons, transgenes, and in particular for its role in paramutation. One of the well studied mutations of the RNA dependent DNA methylation (RdDM) pathway, *mediator of paramutation1 (mop1)*, an RNA-dependent RNA polymerase (RDR), is needed for the establishment and maintenance of paramutation at several maize loci regulating flavonoid biosynthesis. *Unstable factor for orange1* (*Ufo1*) is a dominant mutation of maize which also plays a role in chromatin related gene silencing. *Ufo1* has not been cloned but it has been shown to affect DNA methylation of its reporter gene *pericarp color1* (*p1*). To further characterize the nature of the *Ufo1* mutation, this study has compared *Ufo1-1*, *mop1-1*, single and the *Ufo1-1;mop1-1* double mutants. Phenotypic and molecular characterizations of the effect of each mutation on the paramuted *booster1* (*b1*) allele *B',* as well as genome wide characterization of siRNAs have been undertaken. In addition to *b1* expression, anthocyanin pigment levels were increased in the *Ufo1-1;mop1-1* double mutant as compared to either single mutant. Of 223,044 small RNA (smRNA) clusters expressed across all samples, 13,784 (6.2%) were differentially expressed only in the double mutant. *Ufo1-1* disproportionally affected the expression of smRNA clusters associated with LTR retrotransposons, while *mop1-1* and *Ufo1-1;mop1-1* did not have a particular bias. Both *mop1-1* and *Ufo1-1;mop1-1* expectedly had a larger effect on clusters producing 24nt siRNAs. However, *Ufo1-1* targeted 22nt clusters in addition to clusters which were not associated with a single size class. The additive effect of the double mutation on *b1* expression and pigment accumulation as well as the stark differences of the global siRNA populations suggest that *ufo1*-mediated maintenance of chromatin silencing acts from a pathway separate from the siRNA biogenesis pathway of *Mop1*.

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P321

Examining the transcriptional changes involved in maize centromere inactivation and reactivation

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Functional centromeres are specified by the presence of a kinetochore, a scaffolding structure of protein and RNA that attaches chromatids to spindle fibers during cellular division. RNA transcription of centromeric DNA has been shown to be an important component of functional centromeres in several species. However, specific DNA sequences are neither required, nor sufficient, to direct the recruitment of foundational kinetochore proteins such as CENH3, a centromere-specific histone H3 variant. Thus, centromere activity specification is under epigenetic control.

An intact centromere from the supernumerary maize (*Zea mays*) B chromosome was transferred, via a series of translocations, from the B-A translocation stock TB-9Sb to the short arm of chromosome 9 (9Bic-1), where it immediately inactivated. The inactive B centromere of 9Bic-1 may undergo non-disjunction in the presence of canonical B chromosomes, which can lead to chromosome breakage and a release of the inactive B centromere on a minichromosome. The released B centromere reactivates at a low frequency (0.1%), and has poor heritability. We are able to compare RNA transcription of an active B centromere (TB-9Sb), its inactive descendent (9Bic-1), and two independently derived reactivated B centromeres. RNAseq analysis has revealed striking transcriptional differences between plants harboring active and inactive B centromeres. Interestingly, the two confirmed reactivated centromeres possess markedly different transcript profiles from one another.

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P322

Genetic and epigenetic regulation of maize transcriptome and genome stability under stress conditions: from chromatin modification to lncRNAs and beyond

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Plants have developed complex mechanisms to respond and adapt to abiotic stresses, coupling elaborate modulation of gene expression together with the preservation of genome stability. Epigenetic mechanisms - DNA methylation, chromatin modifications and non coding RNAs - were shown to play a fundamental role in stress-induced gene regulation and may also result in genome destabilization, with the activation and/or the transcription of silenced transposons and retroelements, causing genome rearrangements and novel gene expression patterns.

Maize leaf transcriptome was analyzed by total RNA-Seq in both B73 and *rmr6* (PolIV mutant involved in siRNA biogenesis and in the RdDM pathway) after drought and salt stress application. Reference annotation based transcript assembly allowed the identification both of new expressed loci and splicing variants, improving the current maize transcriptome annotation. Many antisense transcripts matching on the opposite strand of annotated loci were also identified, while more than the 20% of transcripts represent non coding RNA belonging to four classes: siRNAs, shRNAs, lncRNAs and transposable elements (or their relics). Several lncRNAs are modulated by stress application while TE-related sequences are mainly expressed in *rmr6* and up-regulated by the stress.

In parallel we investigated the genome-wide distribution of H3K4me3, H3K9ac and H3K27me3 histone modifications using ChIP-Seq on B73 to explore the epigenomic landascape of drought stress response and adaptation. Stress strongly affects chromatin landscape and a direct correlation between histone modifications and transcription regulation was observed. However, the transcriptional activating modifications H3K4me3 and H3K9ac showed different trends during stress and recovery stage.

Combining these different layers of epigenetic control, we identified a robust list of epigenetic targets, which complete characterization is in progress. Our results clearly showed that to deal with adverse environmental conditions plants have evolved a complex regulatory network and coordinated orchestration of genetic pathways at all levels of epigenetic regulation.

P323

Genetic variation for retrotransposon derived small RNAs in maize

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Retrotransposons comprise a significant proportion of eukaryotic genomes and although typically silent, when expressed they can regulate gene expression in a variety of ways. The majority of the maize genome consists of long terminal repeat (LTR)-retrotransposons that produce a large and diverse source of small interfering RNAs (LTR-siRNAs). Deep sequencing of small RNAs from the seedling shoot apex of 36 diverse maize inbred lines demonstrates that the accumulation patterns of LTR-siRNAs exhibit both a strong genetic component and dramatic genetic diversity. The majority of LTR-siRNAs are produced from a small group of high copy number families that expanded within the maize genome 0.5-1.5 million years ago and are found in all genotypes surveyed; however, the total abundance of 21-22-nt and 23-24-nt LTR-siRNAs for these families varies among genotypes. Other LTR-retrotransposon families produce siRNAs in a subset of genotypes, with examples of some families where siRNAs are only found in one or few inbred lines. We discovered that among a number of factors known to influence population structure among the inbred lines surveyed, divergence in LTR-siRNA profiles was most prominent among genotypes representing populations artificially isolated to exploit hybrid vigor. Quantitative RT-PCR analyses on some of the maize inbred lines and three genetic pedigrees constructed by B73, Mo17 and PH207 showed that mRNA expression level, to some extent, positively related with siRNA abundance in some retrotransposon families, and the mRNA variations existed in those F2 populations were also observed within their further progenies F4 and F6. Our results indicate that LTR-siRNAs contribute another component to regulatory diversity in complex genomes such as maize.

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P324

Identification and transcriptome analysis of a silent allele of *Unstable factor for orange 1*

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A Myb transcription factor encoded by *pericarp color 1 (p1)* regulates phlobaphene biosynthesis in maize. Alleles of *p1* are named after their tissue specific expression pattern, for example, *P1-wr* (white pericarp and red cob glumes) and *P1-rr* (red pericarp and red cob glumes). *Unstable factor for orange 1 (Ufo1)* was first described as a modifier for *P1-wr* expression. In the presence of the dominant mutant *Ufo1-1*, DNA methylation of *P1-wr* is reduced, resulting in increased accumulation phlobaphenes in kernel pericarp, cob glumes and even vegetative tissues. In addition, *P1-wr; Ufo1-1* plants also show pleiotropic defects of plant growth and development, such as reduced plant height, rolled leaves and stunted plants. A silent allele designated here as *Ufo1-1S,* was discovered among the selfed progeny of *Ufo1-1E* (expresser allele) crossed with B73. *Ufo1-1S* showed stable phenotypes resembling wild type *P1-wr; ufo1* plants with colorless pericarp, red cob glumes and normal plant height. DNA methylation of *P1-wr* will be assayed in *P1-wr;Ufo1-1E* and *P1-wr; Ufo1-1S* plants. In order to further elucidate downstream affects *Ufo1-1E* and *Ufo1-1S*, RNA-Seq analysis was performed. Our results indicate that *Ufo1-1S* and wild type transcriptome profiles are more similar to each other than either is to the *Ufo1-1E*. The KEGG pathway enrichment assays showed that flavonoid biosynthesis, starch and sucrose metabolic pathways are up regulated in *Ufo1-1E,* while these show no significant difference in *Ufo1-1S* and wild type.

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P325

Open Chromatin Reveals the Functional Portion of the Maize Genome

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Chromatin accessibility is a highly informative feature of the eukaryotic genome. As recent human ENCODE results demonstrate, assays of open chromatin using DNase hypersensitivity can be used to pinpoint diverse sets of cis-regulatory elements. The discovery of such putative functional regions in crop species has the potential to illuminate the genetic architecture of quantitative traits, as recent data strongly suggests much of the underlying genetic variation resides in the regulatory, non-genic regions of the genome. In this study we use an MNase hypersensitivity assay to discover open chromatin regions within the genome of B73. We show that recombination hotspots within the maize genome correspond to enrichments of open chromatin within nearly all sequence contexts. We also demonstrate that open chromatin is enriched in and around variants explaining quantitative traits, including those far from any known genes. Together, these results suggests that assays of chromatin accessibility will be at least as useful as the transcriptome in defining the functional portion of the genome.

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P326

Over 9,000 new mutants added to UniformMu: 66,000 total Mu insertions with 40% genome coverage

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Over 9,000 new mutants have been added to the UniforMu reverse genetics resource, bringing the total to over 66,000 germinal transposon insertions. These are available in 11,140 independent seed stocks. Approximately 40% of the maize filtered gene set is represented by at least one Mutator allele, and 65% of these genes have two or more mutant alleles. UniformMu insertion lines can be searched by sequence and/or browsed at MaizeGDB.org. Seeds can be requested free of charge through this site or directly from the Maize Genetics Cooperation Stock Center. New Mu insertion locations are released twice per year, once in early Spring and once again in Summer or Fall. Each UniformMu line carries an average of 10 unique, germinal insertions in a W22 inbred background that provides uniform controls for phenotypic comparisons. These lines are provided as segregating F3 material in a “Mu-off”, stable state with a mutable bronze1 allele that serves as a visual marker for activity of the MuDR transposase. Evolving methods and tips for users are available at MaizeGDB.org to aid identification of most useful mutants, requests seed stocks, and strategies for effective tests of co-segregations between genotypes and phenotypes.

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P327

Pericentromeric 22-nt small RNAs suppress heterosis in maize

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A key objective of modern hybrid crop breeding is to increase yield by exploiting heterosis, which relies on the cooperative effect of two distinct genomes. Our work examines the role of small, non-coding RNAs (sRNAs) in establishing heterosis. sRNAs play a major role in modulating the epigenetic landscape and recent publications have hypothesized an sRNA-regulated epigenetic component of heterosis. We think that the mobility of sRNAs, renders them promising candidates for modulating the interplay of diverse genomes in trans – but unequivocal evidence for their contribution to heterosis has been elusive.
Our experimental strategy analyze sRNA expression within the context of a breeding program in a multiplicity of parental genotypes and their hybrid combinations and has enabled the establishment of statistically robust associations between sRNAs and yield heterosis. We sequenced the sRNAs of the 21 inbred lines of a 7 (Flint) x 14 (Dent) factorial and associated these data with the mid-parent heterosis values for grain yield. Our results show that the largest proportion of sRNAs between single inbred combinations of heterotic pools is specific to one or the other inbred line.
In contrast to SNP and mRNA data, differences in sRNA expression between parents showed an overall negative correlation with heterosis. Moreover, sRNAome-based associations revealed a relationship between heterosis and specific parental populations of 22- and 24-nt sRNAs. Importantly, the majority of these sRNAs showed a negative association with heterosis, especially 22-nt sRNAs principally generated from recombination-suppressed, pericentromeric regions. Strikingly, experiments to reduce sRNAome complexity in maize hybrids increased heterotic performance, confirming direct, negative involvement of sRNAs in heterosis. The finding that sRNAs from recombination-suppressed genomic regions play a key role in plant heterosis is likely to impact future plant breeding strategies.

P328

Production and Characterization of a Population of Epigenetic NILs

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The heritability of many phenotypes, especially complex and quantitative traits, is not fully explained by genomic DNA sequence despite the creation of high-density markers. Epigenetic variation might be a source of the "missing heritability" and contribute to the inheritance of complex traits. In maize (*Zea mays*) 85% of the genome is transposable elements which contribute to epigenetic variation by chromatin remodeling, often via small RNAs (sRNAs) and DNA methylation. To understand the epigenetic regulation of phenotypic inheritance, I created a population derived from the B73 inbred line carrying the maize mutant *mediator of paramutation1* (*mop1*). This recessive mutation results in a dysfunctional RNA-dependent RNA polymerase2 (RDR2), and therefore a global decrease in 24-nt sRNA molecules which are usually associated with the maintenance of transposon silencing. Exposing the genome to this condition may generate epigenetic variants in a nearly identical genetic population. A screening in 2014 revealed a high frequency of variants which affected development, metabolism, and pathogen response pathways in the maize plants. In addition, the red fluorescent protein (RFP) was introduced as a reporter gene fused to the tissue-specific promoter for *FLOURY2* (*FL2*) which encodes the abundant alpha-zein seed storage proteins. The population was grown in a nitrogen-deficient nursery to expose active nitrogen-utilization genes to altered regulation via epigenetic variation. The previously stable intensity of RFP shows a wide range of expression variation after the genome has been exposed to *mop1*. Future work will expand this system to utilize the Illinois Long Term Selection Lines, which are divergently selected for high and low protein in the seed. The results to date indicate that these populations will reveal insight into the epigenetic regulation of gene expression and the inheritance of this regulation.

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P329

Reduction of DNA methylation during early embryogenesis enhances growth heterosis of maize plants

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Heterosis is the superior performance of heterozygous hybrid offspring compared to the parental homozygous inbred lines. It has been widely hypothesized to be associated with epigenetic modifications such as DNA methylation. The aim of this work was to test whether DNA methylation pattern established during early embryogenesis impacts upon the heterotic response. We were able to start the treatment of one-day-old maize embryos of inbred lines and hybrids with 5-aza-2'-deoxycytidine (aza), a DNA methyltransferase inhibitor, by developing an *in vitro* embryo sac culture system that allows its controlled application. Methylation sensitive amplified polymorphisms (MSAP) and bisulfite sequencing were performed to show natural and artificially induced methylation dynamics of early embryogenesis, respectively. A successful demethylation of embryonic DNA by aza-treatment was confirmed. Methylation in symmetric CG and CHG contexts was depleted in the early embryo and maintained to the seedling stage. Measuring the growth rates of germinated seedlings was used to determine the effect of methylation inhibition on this key heterotic trait. Comparison of the growth rates between plants from aza-treated embryos and their untreated control group demonstrated a significant increase of growth heterosis upon demethylation. We conclude that DNA methylation patterns established during early embryogenesis of maize have controlling or restraining effects on heterosis.

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P330

Tandem repeats are implicated in both the establishment and maintenance of paramutation at *Pl1-Rhoades*

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Paramutations represent meiotically heritable epigenetic changes facilitated by *trans*-homolog interactions (THI). The maize *purple plant1* locus (*pl1*) regulates anthocyanin production, and one of its alleles, *Pl1-Rhoades*, is a model for understanding the paramutation mechanism. Paramutant *Pl1-Rhoades* states (*Pl′*) are stable, but can revert to a strongly expressed form (*Pl-Rh*) in the absence of **r**equired to **m**aintain **r**epression (RMR) proteins responsible for 24 nucleotide (nt) RNA biogenesis. THIs also stabilize *Pl′* states since hemizygous conditions facilitate reversion to *Pl-Rh*. Other *pl1* alleles are classified by whether they stabilize or destabilize *Pl′* in heterozygous conditions, and these differences are predicted to reflect underlying structural diversity. We used Pacific Biosciences long read sequences to assemble a 200 kb contig representing the *Pl1-Rhoades* haplotype to identify important structural features related to paramutation behaviors. A series of five 2,092 bp tandem repeats (TR) were found coincident with the genetic placement of an essential paramutation element and transcriptional enhancer ~14 kb downstream of the *Pl1-Rhoades* coding region. Another TR previously found ~100 kb upstream of the *B1-Intense* coding region has similar genetic properties, although paramutant *B1-Intense* states do not revert. The *B1-Intense* TR consists of 7 repeats of unique sequence, while the *Pl1-Rhoades* TR includes both LTR retrotransposon, DNA transposon and helitron fragments in addition to a unique 390 bp region. Sequence alignments with *pl1-B73* (stabilizing) and *pl1-Mo17* (destabilizing) implicate the TR unique sequence in maintaining *Pl′* states *in trans*. The presence of 24 nt RNAs at this TR unique region indicate that RMR-based machinery - including RNA polymerase IV - operate on this feature. Together, these new data focus molecular attention to a small region potentially modulated by the THIs responsible for paramutation behaviors at *Pl1-Rhoades*.

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P331

The dynamic changes of genetic imprinting in the progress of maize endosperm development

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Genetic imprinting is a specific epigenetic phenomenon, which mainly occurs to endosperm in plant. Recently, hundreds of imprinted genes had been identified in several plant organisms by high-throughput RNA sequencing, respectively. Our previous study shows that some genes could exhibit imprinted expression in certain specific developmental endosperm stage. However, nearly all genome-wide studies on imprinting identification only used hybrid endosperm of one specific developmental stage. To investigate the dynamic changes of genetic imprinting during the progress of endosperm development, we performed RNA-seq for hybrid endosperm of 4-, 6-, 8-, 10-, 12-, 16-, 24- and 32-DAP from reciprocal crosses of B73 and Mo17 inbred lines. Three known MEGs, *fie1*, *mez1* and *Nrp1*, exhibited maternally expressed in all stages, which certified the accuracy of our data and method. To our surprise, *fie2* exhibited paternally expressed in all stages, which was reported as maternally expressed just in early stages of endosperm. As a result, more than 200 imprinted genes were identified in all stages. Only a part of MEGs and PEGs exhibited short-term imprinted expression among different endosperm developmental stages. Interestingly, more than 92% MEGs with stable imprinting status during the whole endosperm developmental stages possess pDMRs and most of them include maternal preferred H3K4me3 peaks. Correspondingly, PEGs with stable imprinting status during the whole endosperm development tend to possess higher H3K27me3 enrichment. Additionally, we also identified more than 100 lncRNAs, including some antisense MNCs from the intronic region of PEGs. Our results provided a resource about imprinted genes and their allelic expression patterns during the whole development stages of maize endosperm which could be helpful to guide further functional research of imprinted genes.

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P332

The mechanism and impact of alternative transposition-induced DNA re-replication in maize

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The transposable elements *Ac/Ds* often transpose during DNA replication. Transposition from a replicated donor site to an unreplicated target site allows the TEs to replicate twice in a single cell cycle, thereby increasing element copy number. We have shown previously that Alternative Transposition events that involve the termini of two nearby transposons can induce various types of genomic rearrangements; still unknown is the potential impact of coupling DNA re-replication with Alternative Transposition. Here we show that Alternative Transposition-induced re-replication is not limited to the TE sequences, but also extends into the host flanking sequences. The products of these events vary depending upon the initial configuration of the donor elements, the position of the insertion site, the extent of DNA re-replication, and the mechanism of resolution. We have identified a series of inverted and direct duplications, together with the formation of novel Composite Insertions (CIs) at the insertion site (Zhang et al., 2014). To better understand the potential impacts of CIs, we employed a maize stock containing *Ac* and *fAc* (fractured *Ac*) elements inserted into a *p1* allele (*p1-wwB54*) which lacks the first two exons of *p1* and thus specifies colorless pericarp. We screened for mutations that restore pericarp color, hypothesizing that new CIs generated in or near the paralogous *p2* gene may carry regulatory sequences from *p1* required for kernel pericarp expression. From this screen we identified 10 new alleles with colored pericarp; those examined to date contain CIs in *p2* , as predicted. Further structural characterization of the new CIs is underway and will be presented. Our results indicate that Alternative Transposition-induced DNA re-replication may have played an important role in altering genome structure and modifying gene expression during plant genome evolution.

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P333

The role of RdDM chromatin proteins in nucleosome occupancy.

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Epigenetics is the study of heritable changes in gene expression without changes in the underlying DNA sequence. Epigenetic modifications include DNA methylation, covalent histone modifications, nucleosome occupancy, and chromatin structure changes. In plants the RNA-directed DNA methylation (RdDM) pathway has been identified to be involved in *de novo* DNA methylation and gene silencing. While DNA methylation due to RdDM has been extensively studied, little is known about the involvement of chromatin proteins in the pathway. Intriguingly, transcription by plant specific RNA Polymerase (Pol) IV and V in the RdDM pathway is required to prevent transcription by Pol II. One hypothesis to explain this is the presence of chromatin proteins that open up the chromatin in order to facilitate Pol IV and Pol V access; as well as chromatin proteins that are recruited in response to RdDM in order to further repress the target sequence. The role of several RdDM chromatin proteins in nucleosome occupancy has been tested. Micrococcal nuclease (MNase) digested chromatin was tested on transcription start site (TSS) comparative genome hybridization (CGH) microarray at 400 endogenous loci. Our results indicate the ability of these proteins to affect nucleosome occupancy compared to wild type and also compared to the nucleosome occupancy likelihood (NOL) predicted by support vector machines (SVM).

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Transposable Element Polymorphism Impacts Gene Expression in Maize Inbred Lines

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Transposable elements (TEs) comprise the majority of the maize genome, and mutations resulting from TE integration can generate dramatic changes in coding sequence, gene regulation, and phenotype. Despite an acknowledgement of TEs as potential modulators of genome structure and function, intraspecific TE polymorphism in unassembled genomes has been recalcitrant to study, as their repetitive nature complicates examination using short-read sequencing data. To identify TE insertion loci and their genotypes across NAM founder lines, we implement an approach relying on paired-end read mapping and local *de novo* assembly to characterize the allelic state at a locus: whether a TE is present or absent, or no information exists for the locus in that line. TEs are then classified to the level of order for retrotransposons and superfamily for DNA transposons based on structural characteristics like terminal inverted repeats or target site duplications. Despite limitations in identifying TEs in high-copy repetitive regions of the genome, we find thousands of high-confidence insertions per inbred line, most of which represent rare alleles present in a single line. This TE polymorphism contributes to gene expression differences between inbred lines. When a TE is present near or within a gene, expression levels are decreased relative to lines lacking that TE. We find that TE insertion loci provide an additional genotypic characterization of these maize lines, contributing to a growing body of genomic polymorphism data characterizing variation in nonreference maize lines.

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Transposable elements contribute to activation of maize genes in response to abiotic stress

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Transposable elements (TEs) account for a large portion of the genome in many eukaryotic species. Despite their reputation as “junk” DNA or genomic parasites deleterious for the host, TEs have complex interactions with host genes and the potential to contribute to regulatory variation in gene expression. It has been hypothesized that TEs and genes they insert near may be transcriptionally activated in response to stress conditions. The maize genome, with many different types of TEs interspersed with genes, provides a system to study the genome-wide influence of TEs on gene regulation. To analyze the magnitude of the TE effect on gene expression response to environmental changes, we profiled gene and TE transcript levels in maize seedlings exposed to a number of abiotic stresses. Many genes exhibit up- or down-regulation in response to these stress conditions. The analysis of TE families inserted within promoter regions of up-regulated genes revealed that between four and nine different TE families are associated with up-regulated gene expression in each of these stress conditions affecting up to 20% of the genes up-regulated in response to abiotic stress and as many as 33% of not expressed genes activated in response to stress. Expression of many of these same TE families also responds to the same stress conditions. The analysis of the stress-induced transcripts and proximity of the transposon to the gene suggests that these TEs may provide local enhancer activities that stimulate stress-responsive gene expression. Our data on allelic variation for insertions of several of these TEs show strong correlation between the presence of TE insertions and stress-responsive up-regulation of gene expression. Our findings suggest that TEs provide an important source of allelic regulatory variation in gene response to abiotic stress in maize.

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Transposition of a Rice *Mutator*-Like Element in the Yeast *Saccharomyces cerevisiae*

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Mutator-like transposable elements (MULEs) are widespread in plants and are well-known for their high transposition activity as well as their ability to duplicate and amplify host gene fragments. Despite their abundance and importance, few active MULEs have been identified. In this study, we demonstrated that a rice MULE, *Os3378*, is capable of excising and reinserting in yeast, suggesting that yeast harbors all the host factors for transposition of MULEs. The transposition activity induced by the wild-type transposase is low but can be altered by modification of the transposase sequence, including deletion, fusion, and substitution. Particularly, fusion of a fluorescent protein to the transposase enhanced the transposition activity, representing another approach to manipulate transposases. Moreover, we identified a critical region in the transposase where the net charge of the amino acids seems to be important for activity. Finally, transposition efficiency is also influenced by the element and its flanking sequence, *i.e.*, small elements are more competent than their large counterparts. Perfect target site duplication is favorable, but not required for precise excision. In addition to the potential application in functional genomics, this study provides the foundation for further studies of the transposition mechanism of MULEs.

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Using Ac/Ds transposon mutagenesis to characterize the function of maize genes involved in mycorrhizal signaling pathway

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Plants that are capable of forming bacterial nodulation have the distinct advantage of utilizing inorganic nitrogen that is abundant in the air we breathe (nearly 80%). Interestingly, much of the machinery for bacterial nodulation is present in the majority of the flowering plants and used for mycorrhizal symbiosis. The main differences between these two types of symbioses are in early signal perception, and later in activation of downstream genes. In cereals, these processes are still poorly understood. In this study we identified five maize genes that are putatively involved in early and late mycorrhizal symbiosis signaling, including *ZmDMI1 (Pollux)* , *ZmNFR5-1*, *ZmNFR5-2*, *ZmNFP1*, *ZmDMI3*, *ZmNSP1* . To characterize the functions of these genes, we conducted maize mutagenesis using transposable elements *Ac* and *Ds*, which are able to transpose into closely linked genomic region from the original *Ds* donor site. To facilitate large scale screening, we designed a new screening platform and a high throughput genotyping system. To demonstrate the utility of this platform, we conducted a targeted mutagenesis of *ZmDMI1* using a *Ds* donor 57.7kb away and built a tagging population of 4000 individuals. Additional populations are being constructed to tag the genes mentioned above with *Ds* donors 0.5-300kb away from gene targets. We report here the identification of 4 *ZmDMI1 Ds* insertion alleles. In addition, we will discuss the characterization of these mutants and how understanding the function of these genes may serve as the stepping stones for engineering nitrogen-fixation symbiosis in maize.

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CTGA: The CRISPR TALEN genome analyzer

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TALEN and CRISPR technologies have emerged as novel tools for targeted genome editing. The CRISPR TALEN Genome Analyzer (CTGA) is a web-based utility that facilitates the rapid prediction of TALEN and CRISPR constructs in a variety of plant genomes, including maize, including Maize, by quickly identifying target sites for either editing methodology. CTGA takes a user-provided genetic sequence or a user-selected gene model name as an input and identifies potential target sites within the provided sequence. Next, the utility analyzes each potential target site for related off-site targets within a user-selected genome assembly and returns the results. Try it out! The tool is available online at http://ll-cbec-dev.gdcb.iastate.edu/ctga

CTGA was developed by Crop Bioengineering Consortium (CBC), an Iowa State University Presidential Initiative.

Funding acknowledgement: The Crop Bioengineering Consortium at Iowa State University

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Heritable site-specific gene mutagenesis using TALENs in maize

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Transcription activator-like effector nuclease (TALEN) technology has been utilized widely for targeted gene mutagenesis, especially for gene inactivation, in many organisms, including agriculturally important plants such as rice, wheat, tomato and barley. Here we describe application of this technology to generate heritable genome modifications in maize. TALENs were employed to generate stable, heritable mutations at the maize *glossy2* (*gl2*) locus. Transgenic lines containing mono- or di-allelic mutations were obtained from the maize genotype Hi-II at a frequency of about 10% (9 mutated events in 91 transgenic events). In addition, three of the novel alleles were tested for function in progeny seedlings, where they were able to confer the glossy phenotype. In a majority of the events, the integrated TALEN T-DNA segregated independently from the new loss of function alleles, producing mutated, null-segregant progeny in T1 generation. Our results demonstrate that TALENs are an effective tool for targeted genome mutagenesis in maize, to empower the discovery of gene function and the development of trait improvement.

Late Poster Abstracts

P340

Characterization of Low-Dimensional Complex Phenotypes

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Nowadays, there has been a surge in the characterization of complex maize phenotypes. The dimensionality of the phenotypes varies among experiments, but is usually three or greater. As their dimensionality rises, the data become more sparse, so that the number of samples needed sharply increase. Moreover, the number of plants in a group of lines or treatments can be too small to test for an underlying distribution. If the values could be rescaled so that very similar phenotypes could be grouped together, the problem of small sample size could be ameliorated.

Since this problem is very hard to solve exactly without imputing data, we devised a characterization approach to efficiently group three dimensional phenotypes. We first standardize the data to eliminate negative values by a novel method, and then cluster them using MODECLUS, a nonparametric method. The effectiveness of our standardization method is demonstrated by comparison, before and after standardization and nonparametric clustering analysis. We plan to extend this method to higher dimensional data in the future.

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What Can We Learn From Complex Phenotypes?

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Complex phenotypes are both very important and very difficult to dissect. Epistasis, pleiotropy, and interactions with the environment often make experiments hard to interpret. Properly modeling these phenomena for complex or multiple phenotypes is key to accelerating breeding progress and to understanding the nonlinear mappings among genotypes, phenotypes, and their causal mechanisms.

Here we suggest a way of thinking about complex phenotypes we have found helpful. We define a complex phenotype as a function of at least three variables, of which at least one is an input, or independent, variable and at least one is an output, or dependent, variable that one observes. Together, they define a response surface. From the shape of the response surface, it may be possible to deduce an equation that produces it. We call this a "producing function". Even if one cannot immediately deduce a producing function, one can still ask how the response surface changes when the plant is systematically perturbed. The responses produced by different perturbations define a family of mechanistically related surfaces. This family constrains the structure of the producing function, facilitating its deduction. For example, some surfaces may hold one or more variables in the producing function constant, corresponding to a partial derivative. Other perturbations may change the values of coefficients, corresponding to a parametric change. A function that produces each surface in a family defines a process model of the network that generates the complex phenotype.

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