55th Annual Maize Genetics Conference

Program and Abstracts

March 14 – March 17, 2013

Pheasant Run
St. Charles, Illinois

This conference received financial support from:

National Science Foundation
Monsanto
DuPont Pioneer
Syngenta
Dow AgroSciences
BASF Plant Science
KWS
National Corn Growers Association
Biogemma

This conference received other financial support from:

LemnaTec

We thank these contributors for their generosity!

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Cover image description

Selfed colored ear segregating for *a1* (colorless), *su1* (sugary), *y1* (white), *pr1* (red), *sh1* (shrunken), and *wx1* (waxy) in an intense purple popcorn background, showing gene interaction, independent assortment, and linkage.

Cover art by

Dr. Gerald Neuffer
Agronomy Department
University of Missouri

General Information

Meeting Registration

Thursday: 3:00 to 6:00 PM: There will be a table in the Solarium (near main lobby).
9:00 PM to Midnight: 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 PM on Friday and 3-4:30 PM on Saturday. Presenters of even numbered posters should stand by their posters 3-4:30 PM on Friday and 1:30-3 PM on Saturday.

The maize meeting is a forum for presentation and discussion of unpublished material.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

http://maizegdb.org/maize_meeting/2013/downloads.php

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 2013 Steering Committee

Phil Becraft , Chair ..... (becraft@iastate.edu)
Ann Stapleton, co-Chair .... (stapletona@uncw.edu)
Alice Barkan ..... (abarkan@uoregon.edu)
Peter Balint-Kurti..... (peter_balintkurti@ncsu.edu)
Robert Bensen ..... (robert.bensen@syngenta.com)
John Fowler..... (fowlerj@science.oregonstate.edu)
Jinsheng Lai ..... (jlai@cau.edu.cn)
Milena Ouzunova..... (m.ouzunova@kws.com)
Mark Settles ..... (amsettles@ifas.ufl.edu)
Nathan Springer..... (springer@umn.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, Darwin Campbell, and Mary Schaeffer for their tremendous efforts in organizing, assembling, and advertising the conference program. We also thank Angela Freemyer and her team at the University of Missouri Conference Center for helping to organize the conference, handling registration and dealing with a multitude of other issues. Special thanks are also extended to Margy Moore and the Pheasant Run staff for their help in organizing this conference, and to Darwin Campbell for providing AV and other support. Thanks go to Phil Becraft, Robert Bensen, Mark Cigan, Paula McSteen, Milena Ouzunova, Anne Sylvester, and John Fowler 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

2013 Maize Meeting Website
http://maizegdb.org/maize_meeting/2013

Maize Meeting Website (2014 site available November 2013)
http://maizegdb.org/maize_meeting/

Abstract Book (Electronic version)
http://maizegdb.org/maize_meeting/

Cover Image (High-quality color)
http://maizegdb.org/ maize_meeting/coverart/

Upload poster or talk
http://maizegdb.org/ maize_meeting/2013/downloads.php

The MaGNET Program and 2013 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.

2013 MaGNET Awardees

Undergraduate

Beatriz Gomez, University of North Texas
Sean Jackson, Florida A&M University
Leslie Nelson, University of New Mexico

Graduate Student

Eli Borrego, Texas A&M University	Talk #19 (Saturday 9:35AM)
Dale Brunelle, University of North Dakota	Poster #350
Gerald De La Fuente, Iowa State University	Poster #287
Reza Hammond, University of Delaware	
Neil Robbins, Stanford University	Poster #217

Scientist

Lee Bitsoi, Harvard University

Faculty Mentor Accompanying Student

Qunfeng Dong, University of North Texas	Poster #39

The MaGNET program of the Maize Genetics Conference is supported by grant IOS-1302963 from 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 14

10:00 AM – 6:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**
10:00 AM – 3:00 PM	**iPlant Collaborative Workshop** (Utrillo Room)
4:00 PM – 5:00 PM	**MaizeGDB "How to" Tutorials Session #1** (Utrillo Room)
5:00 PM – 6:00 PM	**MaizeGDB "How to" Tutorials Session #2** (Utrillo Room) The two MaizeGDB tutorials are identical. *Pre-registration recommended for the above sessions.*
3:00 PM – 6:00 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: Phil Becraft Pages 23 & 24
7:00 PM	**WELCOME AND ANNOUNCEMENTS** (St. Charles Ballroom) Phil Becraft
7:15 PM	**Mike Scanlon, Cornell University** **[**Plen 1] *Genetics of the maize shoot apical meristem*
8:05 PM	**Julia Bailey-Serres, University of California, Riverside** **[**Plen 2] *Waterproofing plants: Sensing, signaling and response mechanisms*
9:00 PM – 12:00 AM	**REGISTRATION** (Mega Center)
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY** (Mega Center)

Friday, March 15

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 - GENOME STRUCTURE, FUNCTION & EVOLUTION I** Chair: Jinsheng Lai	Talks 1-6. Pages 29-32
8:00 AM	**ANNOUNCEMENTS** Phil Becraft	(St. Charles Ballroom)
8:15 AM	**Fei Lu, Cornell University** *Characterizing structural variation in 19,101 maize inbred lines*	[T1]
8:35 AM	**Wenbin Mei, University of Florida** *The alternative splicing landscape of maize*	[T2]
8:55 AM	**Candice Hirsch, Michigan State University** *Insights into the Maize (Zea mays L.) Pan Genome and Transcriptome*	[T3]
9:15 AM	**Marcela Dotto, Cold Spring Harbor Laboratory** *Analysis of leafbladeless1-dependent small RNAs: new insights into the tasiARFs pathway*	[T4]
9:35 AM	**Hao Wang, University of Georgia** *Twenty to Ten: Centromere Loss and Retention during the Descent of Maize from a Tetraploid Ancestor*	[T5]
9:55 AM	**Lin Wang, Donald Danforth Plant Science Center** *Exploring the mechanism of C_4 photosynthetic differentiation through a unified comparative analysis of maize and rice leaf transcriptomes*	[T6]
10:15 AM	**BREAK**	
10:45 AM – 12:25 PM	**SESSION 3 – QUANTITATIVE GENETICS** Chair: Milena Ouzunova	Talks 7-11. Pages 33-35
10:45 AM	**Eva Bauer, Technische Universität München** *Variation of recombination landscape in European Flint and Dent maize*	[T7]
11:05 AM	**David Wills, University of Wisconsin** *From Many, One: Genetic Control of Prolificacy during Maize Domestication*	[T8]
11:25 AM	**David Hessel, Iowa State University** *Identification of genetic factors conferring resistance to Western corn rootworm beetles, "The Billion Dollar Pest of Maize"*	[T9]
11:45 AM	**Bode Olukolu, North Carolina State University** *A Connected Set of Genes Associated with Programmed Cell Death Implicated in Controlling the Hypersensitive Response in Maize*	[T10]
12:05 PM	**Jianbing Yan, Huazhong Agricultural University** *Genome-wide association study dissects the genetic architecture of quantitative traits in maize*	[T11]

Friday, March 15 (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.

5:00 PM – 6:00 PM **SESSION 4 – GENOME STRUCTURE, FUNCTION & EVOLUTION II**
Chair: Nathan Springer Talks 12-16. Pages 36 & 37

5:00 PM **Tao Zuo, Iowa State University** [T12]
Phenotypic and transcriptional impacts of a specific copy number variation (CNV) in maize

5:20 PM **Mei Zhang, China Agricultural University** [T13]
Genome-wide Single Base Resolution Allele Specific DNA and Histone Methylation Profile in Maize Endosperm

5:40 PM **Gernot Presting, University of Hawaii** [T14]
Getting Hitched: retrotransposons, chromosome movement and centromere drive

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

7:00 PM – 9:00 PM **SESSION 5 – DROUGHT WORKSHOP**
Chair: Bob Bensen Workshop talks 1-3. Pages 27 & 28

7:00 PM **Roberto Tuberosa, University of Bologna** [W1]
Enhancing drought tolerance in maize: Out with the old and in with the new?

7:30 PM **Mark Cooper, DuPont Pioneer** [W2]
Breeding for Drought Tolerance: Discovery to Product

8:00 PM **Daniel Dyer, Syngenta Seeds, Inc.** [W3]
Genetics at the Center of Corn Water Optimization Solutions

8:30 PM **Panel Discussion**

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

Saturday, March 16

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

8:00 AM – 10:15 AM **SESSION 6 – BIOCHEMICAL & MOLECULAR GENETICS**
Chair: Peter Balint-Kurti Talks 15-20. Pages 38-41

8:15 AM **Georg Jander, Boyce Thompson Institute** [T15]
A genetic and biochemical basis for natural variation in maize aphid resistance

8:35 AM **Karl Haro von Mogel, University of Wisconsin-Madison** [T16]
The mapping, genetic analysis, and phenotypic characterization of sugary enhancer1 (se1)

8:55 AM **Marna Yandeau-Nelson, Iowa State University** [T17]
Defining the genetic and metabolic networks responsible for surface hydrocarbon production on maize silks

9:15 AM **Christy Gault, University of Florida** [T18]
Deep mRNA-sequencing of rough endosperm3 seedlings reveals altered splice site usage due to reduced function of splicing factor URP

9:35 AM **Eli Borrego, Texas A&M University** [T19]
Lipid-mediated signaling between maize and pathogens is required for disease development or defense

9:55 AM **Angi Xing, China Agricultural University** [T20]
Map-based cloning and characterization of a maize yellow seedling mutant and its modifier

10:15 AM – 10:45 AM **BREAK**

10:45 AM – 12:25 PM **SESSION 7 – CELL & DEVELOPMENTAL GENETICS**
Chair: Mark Settles Talks 21-25. Pages 42-44

10:45 AM **Thomas Slewinski, Cornell University** [T21]
Origin and Genetics of Kranz Anatomy and C4 Anatomical Specialization

11:05 AM **Amanda Durbak, University of Missouri** [T22]
Transport of boron and water by the tassel-less1 aquaporin is critical for vegetative and reproductive development in maize

11:25 AM **Gokhan Kir, Iowa State University** [T23]
Control of Maize Shoot Architecture by Brassinosteroid (BR) signaling

11:45 AM **Anding Luo, University of Wyoming** [T24]
Warty2 encodes a putative receptor-like Tyr kinase that contributes to maize leaf blade cell expansion

12:05 PM **Michael Pautler, Cold Spring Harbor Laboratory** [T25]
FASCIATED EAR 4 encodes a bZIP transcription factor required for maize meristem size homeostasis

Saturday, March 16 (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: Ed Buckler (St. Charles Ballroom)

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

7:15 PM – 8:55 PM **SESSION 8 – PLENARY TALKS**
Chair: Phil Becraft Pages 25 & 26

7:15 PM **Jen Sheen, Massachusetts General Hospital** [Plen 3]
Glucose Signaling Networks

8:05 PM **James Holland, USDA-ARS, North Carolina State Univ.** [Plen 4]
The diversity of maize

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

Sunday, March 17

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 – NEW RESOURCES & APPROACHES TO MAIZE RESEARCH** Chair: John Fowler	Talks 26-29. Pages 45 & 46
8:20 AM	**ANNOUNCEMENTS** Phil Becraft	(St. Charles Ballroom)
8:30 AM	**Justin Walley, University of California San Diego** *Reconstruction of Protein Networks from an Atlas of Maize Seed Proteotypes*	[T26]
8:50 AM	**Yubin Li, Waksman Institute, Rutgers University** *A sequence-indexed reverse genetics resource for maize*	[T27]
9:10 AM	**Jason Wallace, Cornell University** *The Relative Contribution of Genic and Intergenic Polymorphisms to Natural Phenotypic Variation in Maize*	[T28]
9:30 AM	**Carson Andorf, USDA-ARS** *MaizeGDB: everything old is new again!*	[T29]
9:50 AM	**BREAK**	
10:20 AM – 11:30 AM	**SESSION 10 – NEW RESOURCES & APPROACHES TO MAIZE RESEARCH II** Chair: Ann Stapleton	Talks 30-32. Pages 47-49
10:20 AM	**Christopher Topp, Duke University** *Live 3D imaging reveals the effects of long-term recurrent selection for yield traits on maize root system architecture*	[T30]
10:40 AM	**Manpreet Katari, New York University** *VirtualMaize: A Software platform for translational Systems Biology research in Crops*	[T31]
11:00 AM	**Hilde Nelissen, Flanders Institute of Biotechnology** *Growth Dyn-omics: studying the dynamics of transcriptomics and interactomics within the growth zone of the maize leaf*	[T32]
11:30 AM	**ADJOURNMENT**	

Posters

Computational and Large-Scale Biology

P1	**Hung-Ying Lin** <hungying@iastate.edu>	A Comparison of De Novo Genome Assemblers for Maize
P2	**Alina Ott** <aott@iastate.edu>	A Genome-Wide Analysis of the Expression of Alleles Containing Premature Stop Codons
P3	**Luiz Peternelli** <peternelli@ufv.br>	A novel approach for subset selection of SNP markers for cost-effective implementation of genomic selection
P4	**Lin Li** <lixx1601@umn.edu>	A panoramic view of long non-coding RNAs, their inheritance pattern and genetic mapping in maize
P5	**Natha Miller** <ndmiller@wisc.edu>	Advancing complex phenotype analyses through machine vision and computation
P6	**Michael Freeling** <freeling@berkeley.edu>	Allelic horsepower: An outstanding result from examinations of qTeller-maize two-gene scatterplots
P7	**Matthew Hufford** <mbhufford@ucdavis.edu>	Coevolution of centromeres and foundation kinetochore proteins in the genus *Zea* and the broader Poaceae
P8	**Xianran Li** <lixr@iastate.edu>	Common features of chromosome revealed by systematic analysis across species
P9	**Liliana Andres Hernandez** <landres@langebio.cinvestav.mx>	Comparative analysis of the response to drought stress and recovery irrigation in sorghum and maize
P10	**Ramona Walls** <rwalls@iplantcollaborative.org>	Using Ontologies to Describe Phenotypes in Maize and Across Species
P11	**Lisa Harper** <ligule@berkeley.edu>	The Phenotype RNC Plant Working Group: Ontological Descriptions of Phenotypes Allows Cross Species Comparisons
P12	**Lisa Harper** <ligule@berkeley.edu>	How to Access and Use the New MaizeGDB Website
P13	**John Portwood** <portwoodii@gmail.com>	MaizeGDB has evolved!
P14	**Carolyn Lawrence** <carolyn.lawrence@ars.usda.gov>	Maize Reference Genome Sequence Stewardship: Infrastructure to Enable Rapid Access to Genome Updates and Allow Improved Diversity Representation
P15	**Jacqueline Richter** <jdr1191@iastate.edu>	MaizeGDB Genome Browser
P16	**Emily Mauch** <edmauch@iastate.edu>	Compare Identity By Sequence Relationships of the Ames Diversity Panel using TYPSimSelector
P17	**Ethalinda Cannon** <ekcannon@iastate.edu>	Gene Expression Analysis Tools at MaizeGDB
P18	**Mary Schaeffer** <Mary.Schaeffer@ars.usda.gov>	Pathways at MaizeGDB - Strategies for curation and data sharing
P19	**Taner Sen** <taner.sen@ars.usda.gov>	Metabolic Pathway Resources at MaizeGDB
P20	**Jesse Walsh** <jrwalsh@iastate.edu>	CycTools: An Interface for Exploring and Updating BioCyc Databases
P21	**Kokulapalan Wimalanathan** <kokul@iastate.edu>	Functional annotation of B73 gene models: A machine learning approach

P22	**Kokulapalan Wimalanathan** <kokul@iastate.edu>	A SNP-based high-throughput genetic mapping data analysis tool for mapping mutants and QTL
P23	**Wes Bruce** <wes.bruce@basf.com>	Comparative transcriptomics as a tool for the identification of root branching genes in maize
P24	**Hypaitia Rauch** <hrauch@oakland.edu>	Comprehensive Analysis and Evolutionary Conservation of Alternative Splicing Events of Plant SR Proteins
P25	**Gregory Mathews** <gmathews@berkeley.edu>	Computational identification of conserved root hair elements in maize
P26	**Robert Schaefer** <schae234@umn.edu>	Detecting Causal Genes for Maize Agronomic Traits Using Co-Expression Networks
P27	**Peter Lawson** <pl8210@uncw.edu>	Does combining different detection algorithms improve the robustness of whole-genome prediction when a mixed large and small underlying genetic architecture is present?
P28	**Wenwei Xiong** <xiongwenwei@gmail.com>	DsgMapper: A pipeline tool for the identification of Ds-targeted sequences from next-generation sequencing data
P29	**Ann Meyer** <ameyer@uoguelph.ca>	Estimating allele-specific expression levels from RNA-Seq data
P30	**Chengsong Zhu** <cszhu@iastate.edu>	Estimating the proportion of variation explained by rare variants for six complex traits in whole genome sequence-based studies
P31	**Joshua Stein** <steinj@cshl.edu>	Exploring maize diversity with Gramene
P32	**Allison Karabinos** <askarabin@presby.edu>	Functional analysis of the maize phyllosphere microbiome
P33	**Taoran Dong** <dongtr@uga.edu>	Gene Family Loss and Gain During the Evolution of Flowering Plants
P34	**Zachary Lemmon** <zlemmon@wisc.edu>	Gene Regulatory Change in Maize Domestication
P35	**Michael Carlise** <mcarlise@mix.wvu.edu>	Genome-wide comparative analysis within Sorghum sect. Eusorghum using a next-generation sequencing approach
P36	**Clémentine Vitte** <vitte@moulon.inra.fr>	Genome-wide genetic variation between the French FV2 inbred line and the B73 reference inbred line
P37	**Rute Fonseca** <rute.r.da.fonseca@gmail.com>	Genomic data processing for ancient maize data
P38	**Brett Burdo** <burdo.4@osu.edu>	Grass Gene Regulatory Information Server, GRASSIUS
P39	**Qunfeng Dong** <Qunfeng.Dong@unt.edu>	GSV/mGSV: Web-based Genome Synteny Visualization Tools for Customized Data
P40	**Wenwei Xiong** <xiongwenwei@gmail.com>	HelitronScanner: A two-layered local combinational variable approach to generalized Helitron identification
P41	**Paul Bilinski** <pbilinski@ucdavis.edu>	Historical Changes in Repetitive Sequence and Total Genomic Content In Maize and Related Grasses
P42	**Diane Burgess** <dburgess@berkeley.edu>	Identification and characterization of deeply conserved plant non-coding sequences
P43	**Avimanyou Vatsa** <akvhxd@mail.missouri.edu>	Image Processing and Segmentation of Lesion Mimic Mutants
P44	**Wade Mayham** <wgm343@mail.missouri.edu>	Laboratory Information Management for Computational Experiments
P45	**Jong-Jin Han** <han@cshl.edu>	Large-scale identification of sequence-indexed *Mu* insertion sites in MTM population

P46	**Owen Hoekenga** <owen.hoekenga@ars.usda.gov>	Leveraging non-targeted metabolite profiling via statistical genomics
P47	**Justin Vaughn** <jnvaughn@uga.edu>	Naturally occurring insertions in rice typically create tandem or local duplications that lack a signature of replication slippage
P48	**Michelle Facette** <mfacette@ucsd.edu>	Parallel proteomic and phosphoproteomic analyses define proteotypes of successive stages of maize leaf development
P49	**Sofiane Mezmouk** <smezmouk@ucdavis.edu>	Pattern and distribution of deleterious mutations in maize inbred lines
P50	**Sabarinath Subramaniam** <shabari@berkeley.edu>	Patterns of computed conserved noncoding sequence loss following the paleopolyploidies in the maize and Brassica lineages
P51	**Derek Kelly** <dek343@mail.missouri.edu>	Quantitative Assessment of Complex Visual Phenotypes in Maize Lesion Mutants
P52	**Sanzhen Liu** <liu3zhen@iastate.edu>	Reshuffling of Genic Variation via Meiotic Recombination Generates Novel Gene Expression Patterns
P53	**Matthew Evans** <mmsevans@stanford.edu>	RNA-Seq Analysis of Maize Gametophytic Transcriptomes
P54	**Sheldon McKay** <mckays@cshl.edu>	The iPlant Collaborative's DNA Subway: An Easy-to-Use Tool for Community Annotation of Maize and other Genomes.
P55	**Yinping Jiao** <yjiao@cshl.edu>	The Maize Genome Project, an Update
P56	**Shohei Takuno** <showhey0119@yahoo.co.jp>	The molecular basis of adaptation to highland climates in domesticated maize
P57	**Jeff Glaubitz** <jcg233@cornell.edu>	The reference genome based genotyping-by-sequencing (GBS) bioinformatics pipeline in TASSEL4
P58	**Gregory Downs** <gdowns@uoguelph.ca>	Transcriptional Modules in Maize Development
P59	**Xiao Li** <xiaoli@iastate.edu>	Transcriptomic diversity among maize inbreds
P60	**Toru Kudo** <tkudo@ufl.edu>	UniVIO: a multiple omics database with hormonome and transcriptome data from rice

Biochemical and Molecular Genetics

P61	**Hema Kasisomayajula** <hema090a@gmail.com>	A comparison of MiRNA targets involved in embryo development in maize, sorghum, rice and barley
P62	**Junya Zhang** <zhangjunya@ufl.edu>	A mutation in *DNA polymerase α* co-segregates with a *defective kernel* (*dek*) phenotype in maize
P63	**Rentao Song** <rentaosong@staff.shu.edu.cn>	A new floury endosperm mutant with a mutated z1A 19kD zein gene
P64	**Rentao Song** <rentaosong@staff.shu.edu.cn>	A new transcriptional factor for 19kD α-zein z1A gene family
P65	**Lingling Yuan** <linglingyuan77@gmail.com>	A Novel Functional Genomics Method for Identification of Opaque2 Modifier Genes and Other Genes Involved in Maize Seed Development
P66	**Kevin Chu** <chu16@purdue.edu>	Adult Plant Resistance in the Maize-CCR1 pathosystem: A Biochemical Basis
P67	**Lauren Stutts** <lrs1567@uncw.edu>	Analysis of candidate genes for multiple-stress responses

P68	**John Gray** <jgray5@utnet.utoledo.edu>	Analysis of the role of the ZmMYBGA1 transcription factor in the regulation of amylase genes in maize
P69	**Irene Gentzel** <gentzel.3@buckeyemail.osu.edu>	Assembling the Puzzle: A Closer Look at the Maize Phenolic Pathway Regulators
P70	**Jacob Withee** <witheej@missouri.edu>	Auxin Evo-Devo: Genetic and genomic approaches to understanding the role of auxin in shoot development
P71	**Kyla Ronhovde** <kyla.ronhovde@huskers.unl.edu>	Biochemical Analysis of Kernel Texture and Protein Quality in Maize Endosperm
P72	**Yongrui Wu** <yongrui@waksman.rutgers.edu>	Biogenesis of Protein Bodies in Maize Endosperm
P73	**Christian Caroe** <christiancaroe@gmail.com>	Biomolecular characterization of ancient maize
P74	**Stephanie Locke** <slocke@mail.smcvt.edu>	C-partitioning, defective kernels (deks), and pentatricopeptide-repeat proteins (PPRs)
P75	**Jennifer Derkits** <derkitsjh@vcu.edu>	Canalization at the *r1* Locus in Maize
P76	**Frank Gilcreast** <fgilcreast@mail.smcvt.edu>	Carbohydrate Analysis by Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS)
P77	**Brian De Vries** <bddevries@wisc.edu>	Carbohydrate characterization of the *isa2-339* allele and its interaction with *su1-ref* in maize
P78	**Robert Baker** <bakerrf@missouri.edu>	*Carbohydrate Partitioning Defective1* functions in carbon partitioning and plant defense
P79	**Temitope Salaam** <topesalaam@gmail.com>	Changes in thiamine content in germinating maize lines: the effect of photoperiodic changes
P80	**Brady Barron** <br80bron@gmail.com>	Characterization and mapping of *carbohydrate partitioning defective6*
P81	**Svenja Rademacher** <svenja.rademacher@tum.de>	Characterization of drought stress response in two elite maize lines
P82	**Mo Jia** <Mo_Jia@baylor.edu>	Characterization of Maize Eukaryotic Translation Initiation Factor 5A Reveals Association with an Actin-Rich Cytoskeletal Fraction
P83	**Alan Myers** <ammyers@iastate.edu>	Characterization of maize genes encoding plastidial ADPglucose pyrophosphorylase
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P109	**Oyenike Adeyemo** <adeyemona@gmail.com>	Genetic diversity assessment among yellow endosperm tropical-adapted maize inbred lines using SSR and allele specific PCR-based markers
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P130	**Shan Jin** <szj133@psu.edu>	Maize nested-association-mapping (NAM) founder lines exhibit diverse responses to caterpillar feeding
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Fast-Flowering Mini-Maize: Seed to Seed in 60 Days

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P235 **Christopher Bottoms** <bottomsc@missouri.edu>
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P237 **Brent Buckner** <bbuckner@truman.edu>
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The Maize Germplasm Bank at CIMMYT : An Invaluable Genetic Resource for Maize Geneticists, Breeders, Producers and Consumers throughout the World

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P270	**Solomon Fekybelu** <Solomon.Fekybelu@daff.qld.gov.au>	Genetic variability in tropical maize breeding populations subjected to a reciprocal recurrent selection program
P271	**Junping Chen** <junping.chen@ars.usda.gov>	Genetic variation for high temperature tolerance in maize
P272	**Lian Lian** <lian0090@umn.edu>	Genomewide prediction accuracy within 1000 biparental maize populations
P273	**Amy Jacobson** <jaco0795@umn.edu>	Genomewide prediction within an untested biparental cross
P274	**Jason Morales** <jasonmorales@purdue.edu>	Genomic Approaches for Improving Grain Yield in Maize Using Formerly Plant Variety Protected Germplam
P275	**Timothy Beissinger** <beissinger@wisc.edu>	Genomic impact of artificial selection for number of ears per plant in maize
P276	**Xin Li** <xinli@iastate.edu>	Genotype by Environment Interaction of Sorghum Flowering Time
P277	**Robert Elshire** <rje22@cornell.edu>	Genotyping-By-Sequencing (GBS): Building A Resource for the Maize Community
P278	**Kelly Swarts** <kls283@cornell.edu>	Genotyping-by-sequencing the genomic imprints of temperate adaptation in maize landraces from the southwestern United States and northern Mexico
P279	**Felix Frey** <frey@mpipz.mpg.de>	Heat tolerance of maize - Improving the tolerance by next generation plant science
P280	**Zongliang Chen** <zchen@cau.edu.cn>	High resolution QTL detection using large F2 population and high throughput genotyping in maize
P281	**Stefan Schwartz** <stefan@lemnatec.de>	High Throughput Phenotyping - A boost for genomics in the 21st century
P282	**Alexander Lipka** <ael54@cornell.edu>	High-resolution mapping of tocochromanol and carotenoid grain traits via NAM-GWAS reveals distinct genetic architectures in maize
P283	**Jinliang Yang** <yangjl@iastate.edu>	Identification and Validation of Maize Loci Controlling a Yield Component Trait via GWAS
P284	**Race Higgins** <racehggns@gmail.com>	Identification of Sorghum Height and Maturity QTL in Nearly Isogenic BiparentaI Populations
P285	**Peter Balint-Kurti** <peter_balintkurti@ncsu.edu>	Identification of southern leaf blight resistance genes by genome wide association study
P286	**Bhornchai Harakotr** <harakotr@iastate.edu>	Inheritance of anthocyanin concentration in purple waxy corn (Zea mays L.) kernel and cob
P287	**Gerald De La Fuente** <gerald@iastate.edu>	Investigating the Maternal Genetics of Haploid Induction Rate

P288	**Christine Lucas** <cjlucas@illinois.edu>	Investigating the regulation of grain protein concentration in the Illinois Protein Strain Recombinant Inbreds: Precision phenotyping using *floury2*-mRFP as an alternative to NIR
P289	**Martin Garcia-Flores** <masterfoodscience@live.com>	Linear regression model to predict the agronomic performance of maize plants
P290	**Aaron Lorenz** <alorenz2@unl.edu>	Mapping QTL controlling tolerance to Goss's bacterial wilt of maize
P291	**Zhengbin Liu** <liuzhen@missouri.edu>	Mapping QTLs with Maize-Teosinte NILs
P292	**Carrie Thurber** <cthurber@illinois.edu>	Mining for biofuel gold: Introgression mapping of maturity and height loci in sorghum
P293	**Heather Manching** <hkm8595@uncw.edu>	Nonlinear effects of abiotic and biotic stress on yield and phyllosphere diversity
P294	**Lisa Marie Krchov** <krcho001@umn.edu>	Multi-environment validation experiments to assess the accuracy of phenotypic and genomewide selection within biparental doubled haploid breeding populations
P296	**Aaron Lorenz** <alorenz2@unl.edu>	Optimal resource allocation for a maize genomic recurrent selection program
P297	**Jason Peiffer** <japeiffe@ncsu.edu>	Optimizing sampling for estimation of genetic architecture and prediction of phenotypes
P298	**German Muttoni** <muttoni@wisc.edu>	Phenotypic and Genetic Dissection of Maize Internode Length
P299	**Catherine Rutledge** <clrutledg@presby.edu>	Phenotypic Response to A. avenae Infection in the IBM 94 Maize Population
P300	**Srinivasa Chaluvadi** <src@uga.edu>	Plant genetic contributions to microbial colonization in the rhizospere and roots of several panicoid grasses
P301	**Aniruddha Acharya** <aniruddha1302@gmail.com>	Production of biofuel from cellulosic biomass
P302	**Sylvia Morais de Sousa** <sylvia.sousa@embrapa.br>	QTL mapping for P efficiency and root traits under low phosphorus availability in maize and identification of putative PSTOL1 homologues
P303	**Yijun Wang** <yjwang61@163.com>	QTL mapping of resistance to Aspergillus flavus infection in maize (Zea mays L.)
P304	**Brittany Glaza** <glaza@wisc.edu>	Reciprocal Differences in the Expression of *Corngrass1* (*Cg1*)
P305	**Frederike Horn** <horn@mpipz.mpg.de>	Resistance to barley yellow dwarf virus in segregating populations of maize
P306	**Sylvia Morais de Sousa** <sylvia.sousa@embrapa.br>	Root morphological analysis of a maize diversity panel under low and high phosphorus
P307	**Sylvia Morais de Sousa** <sylvia.sousa@embrapa.br>	Root morphology comparison between maize recombinant inbred lines population per se and crossed with a common tester under low phosphorus condition
P308	**Dhyaneswaran Palanichamy** <dp429@cornell.edu>	Screening maize (Zea mays L.) germplasm for the crtRB1 and LcyE polymorphisms to increase β- carotene content in Indian conditions..
P309	**Soheil Zarandy** <s_zarandy@yahoo.com>	Stepwise regression for grain yield of maize hybrids under drip irrigation
P310	**Mohammad Dakhili** <dr_dakhili@yahoo.com>	Study correlation analysis between characters in maize hybrids in normal condition

P311 **Soheil Zarandy** <s_zarandy@yahoo.com> — Study correlation analysis between characters in maize hybrids under drip irrigation

P312 **M.H. Gharib Mojeni** <hasangharib@yahoo.com> — Study on effects of drought stress and mycorrhizal fungi on water use efficiency in corn silage

P313 **Jasmine Freeman** <jasminefreeman87@gmail.com> — The impact of recombination on allelic expression in the *Bz1/Sh1* Interval of Zea mays

P314 **Clément BUET** <clement.buet@biogemma.com> — The molecular characterization of a MAGIC population reveals high potential for gene discovery

P315 **Claudia Irene Calderón** <cicalderon@wisc.edu> — Towards the fine mapping of a major QTL controlling the number of rows of kernels per ear

P316 **Peter Bradbury** <pjb39@cornell.edu> — Using GBS data to study the distribution of recombination breakpoints in two maize NAM populations

P317 **Matthew Murray** <mdm266@cornell.edu> — Using NAM to put a dent in our understanding of maize kernel type

P318 **Jessica Bubert** <jbubert2@illinois.edu> — Using QTL Enrichment to Improve Nitrogen Utilization in Maize

Transposons & Epigenetics

P319 **Gustavo Rodriguez** <gustavorg2306@hotmail.com> — A reverse screen to identify maize genes involved in the response to phosphorus starvation

P320 **Martha Ibore** <mibore@iastate.edu> — AC induced rearrangements in the maize p1 gene

P321 **Yubin Li** <yubin@waksman.rutgers.edu> — All in one: *TED*, a single gene encodes the transposition functions of a novel autonomous element of the *Mutator* superfamily

P322 **Brian Lynch** <btlynch@oakland.edu> — Alternative usage of splice sites augments the transcript diversity of Helitron captured genes between different maize inbred lines

P323 **Dhanushya Ramachandran** <dhanushhya@gmail.com> — Comparative analysis of LTR-retrotransposons in *Sorghum bicolor* and its perennial relative *S. propinquum*

P324 **Stephanie Haase** <sjhaase@iastate.edu> — Does the Petunia dTph1 Element Undergo Alternative Transposition?

P325 **Cristian Forestan** <cristian.forestan@unipd.it> — Epigenetic regulation of maize transcriptome and TEs activity in response to environmental stresses

P326 **Shaojun Xie** <xieshaojun0621@cau.edu.cn> — Epigenetic variation through the breeding processes of maize

P327 **John Laurie** <johndlaurie3@gmail.com> — Epigenetics, the cell cycle and the origin of endosperm

P328 **Steven Eichten** <eicht021@umn.edu> — Genetic and Epigenetic Control of DNA Methylation Variation in Maize

P329 **Alyssa Bagadion** <bagadiona@ufl.edu> — Genetic and phenotypic characterization of the *maternal rough endosperm1* (*mre1*) locus

P330 **Amy Sloan** <sloan@bio.fsu.edu> — Genetic mapping of *transgene reactivated mutant 1 (tgr1)*, a novel allele of the largest subunit of RNA Polymerase IV in maize

P331 **Patrick West** <west0845@umn.edu> — Genome wide H3K9me2 methylation profiles in maize highlight associations with DNA methylation

P332 **Amanda Waters** <water157@umn.edu> — Imprinting is highly conserved among maize haplotypes

P333	**Jonathan Gent** <gent@uga.edu>	Interactions between methylation pathways in intergenic chromatin regulation
P334	**Ryan Douglas** <DouglasRN@missouri.edu>	Investigating the epigenetic specification of maize centromeres
P335	**Nathanael Ellis** <nellis@plantbio.uga.edu>	Investigating the importance of MITEs as insulators against heterochromatin spreading
P336	**Tzuu-fen Lee** <tzuufen@udel.edu>	Maize *Ufo1* modulates tissue-specific small RNA profiles and locus-specific gene expression
P337	**Kara Dragone** <DragoneK@duq.edu>	Methylation Patterns of the Maize *R-stippled* Derivative Lines
P338	**Elizabeth Buescher** <ebuesche@purdue.edu>	MOP1 impacts the maternal contribution to maize seed
P339	**Damon Lisch** <dlisch@berkeley.edu>	Mu killers, old and new
P340	**Dongyan Zhao** <zhaodon4@msu.edu>	Nested Insertions and Accumulation of Indels in Coding-MULEs (*Mutator*-like Transposable Elements) are Negatively Correlated with Abundance of MULEs in Maize and Rice
P341	**Thelma Madzima** <tmadzima@bio.fsu.edu>	Paramutation-like interactions between two transgenes in maize leads to cytosine hypermethylation and homology dependent silencing
P342	**Fang Bai** <fbai001@ufl.edu>	Parent-of-origin effect seed mutants from UniformMu transposon tagging population in maize
P343	**Anthony Studer** <astuder@danforthcenter.org>	Regional mutagenesis of a tandemly duplicated *carbonic anhydrase* gene cluster using the maize transposable elements *Ac*/*Ds*
P344	**Jennifer Rundquist** <jrundquist02@hamlineuniversity.edu>	Relationships between H3K27me3 Modifications and Gene Expression
P345	**Jay Hollick** <hollick.3@osu.edu>	*required to maintain repression5* encodes a DICER-LIKE3 isoform required for both 24nt RNA biogenesis and for paramutation
P346	**Qing Li** <cauliqing@gmail.com>	Small RNAs Contribute to Gene Expression Divergence and Inheritance in Maize Hybrids
P347	**Li Li** <lli1204@iastate.edu>	The glossy13 gene encodes a putative ABC transporter
P348	**Linda Stroud** <lstroud@bio.fsu.edu>	The initial characterization of *chr120* point mutation alleles
P349	**Joy-El Barbour** <joy-el.barbour@berkeley.edu>	The maternal epigenome influences small RNA profiles of its progeny
P350	**William F Sheridan** <william.sheridan@und.edu>	The Presence of Activator (Ac) Elements in Nonspotted Kernels Produced by Transposition of Seven Maize Ac Elements Located on the Short Arm of Chromosome 1
P351	**Nur Suhada Abu Bakar** <nxa155@psu.edu>	Transgenerational inheritance of epigenetic regulation by *Unstable factor for orange1 (Ufo1)* in maize
P352	**Amanda Costa** <acosta3@mail.smcvt.edu>	UniformMu insertions in gene for Exocyst 70 subunit correlate with empty pericarp phenotype
P353	**Jay Hollick** <hollick.3@osu.edu>	Global run-on sequencing identifies transcriptional control of the maize genome by RNA polymerase IV
P354	**Dafang Wang** <dwang@iastate.edu>	Inverted duplication alleles generated by Ac-induced Sister Chromatid Transposition (SCT) at p1 locus show repressed Ac activity and reduced levels of Ac transcript

Plenary Talk Abstracts

Plenary 1 **Thursday, March 14 7:15PM**

Genetics of the maize shoot apical meristem

(presented by Mike Scanlon <mjs298@cornell.edu>)
Full Author List: Scanlon, Michael J[1]; Johnston, Robyn[1]; Leiboff, Samuel[1]; Javelle, Marie[2]; Li, Lin[4]; Li, Xianran[3]; Muehlbauer, Gary J[4]; Petsch, Katherine A[2]; Schnable, Patrick S[3]; Thompson, Addie[4]; Timmermans, Marja C P[2]; Yu, Jianming[3]
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The maize vegetative shoot apical meristem (SAM) is a complex signaling network comprising an indeterminate pool of stem cells that is immediately juxtaposed to leaf initial cells. Arising de novo early in embryogenesis, the SAM is responsible for the development of all above ground organs in the plant and must maintain a precise equilibrium during which cells lost to newly-initiated leaves are replenished by stem cells that divide to maintain the SAM. Widespread variations in shoot meristem structure have evolved among land plant lineages. Examples include the single-celled apices in the moss *Physcomitrella patens*, the multicellular meristems with prominent apical cells in lycophytes such as *Sellaginella moellendorffii* and the histologically stratified shoot meristems of angiosperms like maize (*Zea mays*). Even within the genus *Zea*, a wide range of SAM morphometric diversity is observed. Despite this variation in shoot apical structure, meristem function during lateral organ initiation and stem cell renewal is conserved throughout plant evolution. Our recent work utilizes genetic and genomic approaches in combination with a variety of imaging technologies toward understanding the shared and divergent genetic networks regulating SAM structure and function in maize and model land plants.

Funding acknowledgement: National Science Foundation (NSF)

Waterproofing plants: Sensing, signaling and response mechanisms

(presented by Juila Bailey-Serres <serres@ucr.edu>)
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Floods are increasingly responsible for major crop losses due to climate change and demands for food and feed production. Flooding stress, including soil waterlogging and partial to complete submergence, reduces oxygen availability for mitochondrial ATP production, triggering alterations in gene transcription, mRNA translation and energy metabolism. The formation of root aerenchyma is an adaptation to waterlogging that is constitutive in rice and inducible in maize. Nonetheless, cultivated maize dies within days of sudden submergence of aerial tissue, in marked contrast to rice and Arabidopsis. Recently, the plant-specific group VII Ethylene Response Factor (ERF) transcription factors have emerged as pivotal regulators of flooding and low oxygen responses. In rice, the group VII ERFs SUB1A and SNORKEL1/2 enable survival or escape of submergence, respectively. The turnover of the five Arabidopsis group VII ERFs of is oxygen-mediated, limiting their accumulation under hypoxic conditions. These transcription factors regulate many of the genes encoding the metabolic enzymes first identified by Marty Sachs and Michael Freeling as the “anaerobic polypeptides” of maize. My talk will describe our current understanding of low oxygen sensing, signaling and response gleaned from rice and Arabidopsis. As B73 maize encodes several low-oxygen induced Group VII ERF genes with characteristics of the oxygen-destabilized Arabidopsis orthologs, it may be feasible to leverage conservation in oxygen sensing and response mechanisms to improve flooding survival in maize.

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

Glucose Signaling Networks

(presented by Jen Sheen <sheen@molbio.mgh.harvard.edu>)
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Glucose fuels life and is a central nutrient signal for growth regulation in a broad range of organisms from bacteria, yeasts to plants and humans. Despite the essential and multifaceted regulatory roles of glucose in gene expression, physiology, metabolism, cell proliferation, growth and development, and human diseases, the molecular and cellular mechanisms of glucose signaling remain elusive in multicellular plants and animals. Our research in plants has provided compelling molecular, chemical, genetic and genomic evidence that hexokinase1 (HXK1) and target-of-rapamycin (TOR) kinase are two evolutionarily conserved master regulators in glucose signaling, which integrate direct glucose sensing and glucose-driven energy signaling to orchestrate transcriptional networks and plant growth in response to environmental cues. Our recent findings uncover two surprising and distinct functions of specific HXK1 that mediate glucose signaling without its catalytic activity. In leaves, HXK1 senses excess glucose at low nitrate and acts in the nucleus to modulate transcriptional reprogramming. In nitrate sufficient conditions, HXK1 plays an additional novel function in promoting cell and organ size and growth. We have also developed new chemical genetic tools to discover a previously unrecognized central role of glucose-TOR signaling in controlling stem/progenitor cell proliferation in meristem establishment and postembryonic plant growth. The uncovering of the novel glucose-HXK1 and glucose-TOR signaling mechanisms will establish new paradigms in glucose responses and regulations in plants and animals, and build a new conceptual framework to enhance our understanding of the molecular and cellular mechanisms of glucose signaling from plants to humans.

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

The diversity of maize

(presented by James Holland <Jim.Holland@ars.usda.gov>)
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The genus *Zea* is tremendously variable. Even within the domesticated species maize, variation is abundant: from DNA sequences (SNPs, indels, transposon organization, and higher level structural variation) to visible and otherwise measureable phenotypes. Why does the species maintain such a high level of genetic and phenotypic variation? Are sequence and phenotypic variations partitioned in similar ways, and what is their relationship? High levels of diversity complicate maize genetics in many ways, but also present incredible opportunities for genetic analysis. The diversity of Zea includes many deleterious genetic variants, but winnowing with genetics tools and breeding methods can help identify useful alleles and germplasm sources outside of current elite breeding pools.

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

Drought Workshop Abstracts

Workshop 1 **Friday, March 15 7:00PM**

Enhancing drought tolerance in maize: Out with the old and in with the new?

(presented by Roberto Tuberosa <roberto.tuberosa@unibo.it>)
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Although phenotypic selection has been effective in enhancing maize yield under drought conditions, genomics-assisted breeding (GAB) via marker-assisted selection (MAS) or genome-wide selection (GWS), and genetic engineering (GE) are being increasingly adopted to improve drought tolerance. Enhancing maize productivity in low-moisture conditions through approaches targeting one or only a few loci via MAS or GE is a daunting undertaking due to the quantitative inheritance and low heritability of the traits governing the adaptive response to drought and, ultimately, yield. Accordingly, the elusive nature of the relevant QTLs and the marked context-dependency of their effects further limit their exploitation in breeding programs. Hence, a critical factor for a more widespread adoption of MAS and GE for improving drought tolerance is the difficulty in identifying loci characterized by consistently large effects across different elite genetic backgrounds, environments and management practices. The increased availability of SNPs and sequencing data facilitate the discovery of drought-adaptive loci via association mapping (e.g. NAM populations). Growing interest is being devoted to modeling yield under different water regimes based on QTL effects for drought-adaptive, morpho-physiological features (e.g. root architecture, leaf growth, etc.) and environmental variables. Examples will be presented and critically appraised.

From a breeding standpoint, GAB has recently provided major breakthroughs as shown by the commercial release of drought-tolerant maize hybrids selected via GWS or targeting specific loci via MAS. While QTL cloning will increasingly shed light on the molecular and functional basis of drought tolerance, accurate and relevant phenotyping remains a major challenge for more effectively leveraging GAB and GE. Adequately meeting this challenge requires access to managed-drought stress nurseries, a deeper understanding of the factors limiting yield in drought-stressed maize and a multidisciplinary approach.

Funding acknowledgement: The financial support of the EU-funded project DROPS (DRought-tolerant yielding PlantS; FP7-244374), Pioneer-DuPont and KWS is gratefully acknowledged

Workshop 2 **Friday, March 15 7:30PM**

Breeding for Drought Tolerance: Discovery to Product

(presented by Mark Cooper <mark.cooper@pioneer.com>)
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Germplasm, genetics, phenotyping and selection, combined with a clear definition of product targets are foundational to successful plant breeding. Manipulating these resources, plant breeders play a large numbers game. Today, molecular technologies enable detailed views of plant genomes and provide unprecedented access to sequence data, opening new ways to study trait genetic architecture. While the numbers game will continue to be a feature of plant breeding for the foreseeable future, genetic understanding of the traits necessary for successful hybrid products in the target environments is enhancing our ability to utilize prediction methodology. These capabilities have been implemented to enable the creation of maize hybrids with improved levels of drought tolerance. Lessons learned and future opportunities for further improving the drought tolerance of maize will be discussed.

Workshop 3 **Friday, March 15 8:00PM**

Genetics at the Center of Corn Water Optimization Solutions

(presented by Dan Dyer <dan.dyer@syngenta.com>)
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According to the United Nations, by 2025, there will be another billion people on Earth. That will create the demand for another 350 million metric tonnes of grain. Maize must provide a larger portion of that than any other foodstuff. The leading limitation to our ability to meet this demand is water availability. Addressing drought tolerance in maize is simply fundamental to our ability to feed ourselves. Yet, there is no more complex challenge undertaken to date in agriculture. This challenge clearly demands integrated cropping solutions. In this talk, we will explore the current understanding of the role of genetics in these cropping solutions, including the impacts of breeding and genetic modification, the integration of genotyping and phenotypic selection, and the challenge of understanding genotype-environment interactions in the context of cropping strategies.

Funding acknowledgement: Syngenta and the Syngenta Foundation for Sustainable Agriculture

Short Talk Abstracts

SESSION 2 - GENOME STRUCTURE, FUNCTION & EVOLUTION I
Chair: Jinsheng Lai Friday, March 15. 8:15 AM – 10:15 AM

T1
Characterizing structural variation in 19,101 maize inbred lines

(presented by Fei Lu <fl262@cornell.edu>)
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The highly variable genome of maize is saturated with structural variation, including copy number variation (CNV), presence and absence variation (PAV), translocations, and inversions. In addition to its important role in shaping genomic architecture, this structural variation may have a huge impact on phenotypic variation in maize. Employing a genotyping-by-sequencing (GBS) protocol using the restriction enzyme ApeKI, we sequenced 19,101 maize inbred lines, generating about 17.2 billion reads in total. Using a genetic mapping approach (GWAS and joint linkage mapping versus 681,257 GBS SNPs), 4.4 million GBS tags were mapped as presence/absence markers with high precision, with >95% of the B73 (positive control) tags mapped to within 1 Mb of their true position. About 1.4 million tags were identified as insertion PAVs not present in the B73 reference genome. Using a 200 Kb bin-based read depth approach, 3,263 and 7,898 bins had significantly lower (deletion PAV) or higher (CNV) read depth ratios than B73, respectively. Both the PAVs and CNVs were distributed throughout the genome, but were more concentrated in pericentromeric regions. The prevalence of structural variation was positively correlated with repeat density and negatively correlated with recombination rate and gene density. Genomic bins with high amounts of structural variation were enriched for genes involved in stress response. In contrast, genes involved in biosynthetic and metabolic processes, or interacting with organelles, were underrepresented. Using the identified bin-based PAV markers, GWAS was performed for 30 traits in both the nested association mapping (NAM) population and an association mapping population consisting of 3000 maize lines from the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. Further analysis of the GWAS results, currently in progress, will be presented.

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

T2
The alternative splicing landscape of maize

(presented by Wenbin Mei <wmei@ufl.edu>)
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Alternative Splicing (AS) produces multiple isoforms from a single pre-mRNA through selective use of splice sites. AS is known to play roles during development, stress response, and flowering by influencing protein diversity or affecting protein levels by regulating message processing. However, the extent of AS in plants is not well understood. Despite having a sequenced genome and being a key crop, little is known about genome-wide patterns of alternative splicing in maize. We have identified haplotype- and tissue-specific patterns of alternative splicing via analyses of extensive RNA-seq data sets from multiple tissues of B73 and Mo17. Examination of splicing patterns in the reciprocal hybrids of these lines reveals parent-of-origin impacts on splicing patterns. Using the intermated B73xMo17 recombinant inbred lines (IBM RILs) we have identified cis- and trans-acting regulatory variation (sQTL) that affects AS. Some of the trans-sQTL overlap chromosomal positions of genes that encode splicing factors. We are now testing whether allelic expression levels of splice junctions exhibits dominant or additive modes of actions, analyzing the epigenetic features around splice sites and mapping differences in AS between the two subgenomes of maize.

T3

Insights into the Maize (*Zea mays* L.) Pan Genome and Transcriptome

(presented by Candice Hirsch <hansey@msu.edu>)
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Within a species there are genes that are present in every individual termed the core genes and those that are only present in a subset of the individuals termed the dispensable genes. Core and dispensable genes are collectively termed pan genes. Dispensable genes have been shown to be important in environmental adaptation and phenotypic variation. We conducted sequencing of whole seedling RNA from a set of 503 diverse maize inbred lines to evaluate the maize seedling pan-transcriptome as a proxy to the maize pan genome. Reads unmapped to the B73 reference sequence were identified and a *de novo* assembly of equally representative reads from each line identified 8,681 novel representative transcript assemblies (RTAs). BLAST alignments to the maize PlantGDB-assembled unique transcripts, Rice Proteins, Sorghum Proteins, and UniRef100 revealed support for approximately half of these sequences. For the B73 reference genes, 37.9% of the genes were expressed in all of the lines and 46.5% were expressed in a subset of the lines, while for the RTAs only 16.4% were expressed in all of the lines and 82.7% were expressed in a subset of the lines. Linkage disequilibrium mapping with ~500K single nucleotide polymorphisms (SNPs) placed 76.7% of the RTAs with at least one SNP (4,429) to a single position within the maize B73 reference sequence. The positioned RTAs were distributed throughout the entire genome with lower numbers observed in centromeric regions. Individual assemblies were generated from 366 of the lines with greater read depth, and sequence-based clustering was used to assess the open/closed nature of the maize pan genome. Stepwise addition of lines from 2 to 366 lines showed a plateau in the total number of orthologous groups/singletons with a maximum of 24,129, demonstrating that the maize pan genome, as estimated by the seedling transcriptome, is a closed genome.

Funding acknowledgement: Department of Energy (DOE)

T4

Analysis of *leafbladeless1*-dependent small RNAs: new insights into the tasiARFs pathway

(presented by Marcela Dotto <dotto@cshl.edu>)
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Maize LEAFBLADELESS1 (LBL1) and *Arabidopsis* SUPPRESSOR OF GENE SILENCING3 (SGS3) play orthologous roles in the biogenesis of 21 nucleotide trans-acting short-interfering RNAs (tasiRNAs). The phenotypes conditioned by mutation of *lbl1* and *sgs3* are, however, strikingly different, suggesting that the activities of these small RNA biogenesis components or the tasiRNAs and their targets might not be entirely conserved. To investigate the basis for this phenotypic variation, we compared the small RNA content between wild type and *lbl1* seedling apices. Besides the 21-nt fraction of small RNAs, 22- and 24-nt long small RNAs were also found to be downregulated in *lbl1*. Small RNAs showing significant differential accumulation map to a diversity of genomic loci, revealing unexpected genome-wide links between LBL1, miRNAs, and retrotransposon- and DNA transposon-derived siRNAs. We further identified genomic regions generating phased siRNAs, including numerous loci generating 22-nt phased small RNAs not previously described in other plant species. Finally, this analysis identified a total of nine tasiRNA precursor *(TAS)* loci. All contain two miR390 target sites and eight have the potential to generate one or more copies of the evolutionary conserved tasiARF, indicating all nine *TAS* loci are members of the *TAS3* family. No *TAS* loci triggered by 22-nt miRNAs were identified. A combination of target prediction, RNAseq and degradome analysis indicates that the tasiARFs are the only functional tasiRNAs in the maize vegetative apex where they regulate expression of *arf3* homologs. Together these data indicate that divergence in ARF3 function could account for the dramatic phenotypic differences observed upon mutation of *sgs3/lbl1* in *Arabidopsis* and maize.

Funding acknowledgement: Dupont-Pioneer

T5

Twenty to Ten: Centromere Loss and Retention during the Descent of Maize from a Tetraploid Ancestor

(presented by Hao Wang <wanghao@uga.edu>)
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Although centromere function is highly conserved in eukaryotes, centromere sequence organization in plants has been shown to be highly variable. Conserved single-copy pericentromeric nucleotides (CPNs) of sorghum and maize were found to be diagnostic characteristics of adjacent centromeres. By analyzing comparative map data and centromeric/pericentromeric sequences of sorghum, maize and rice, the primary evolutionary events related to centromere dynamics were discovered for the maize lineage after its divergence from its common ancestor with sorghum. (i) Remnants of ancient centromeric/pericentromeric regions were found for the 10 lost ancestral centromeres, indicating cases where both ortholgous centromeres were retained from some ancestral chromosomes and cases where both orthologous centromeres were lost. (ii) Five cases of long distance, intra-chromosome movement of centromeres/pericentromeres were detected in the retained centromeres, with inversion the major process involved. (iii) The 12 major chromosome rearrangments that led to maize chromosome number reduction from 20 to 10 were uncovered. (iv) Besides chromosome insertion near (but not always into) other centromeres, translocation and fusion were also found to be important mechanisms underlying maize chromosome number reduction. (v) Comparison of chromosome structures confirmed that maize is derived from a recent tetraploid ancestor.

Funding acknowledgement: National Science Foundation (NSF), Georgia Research Alliance, Giles Professorship at the University of Georgia

T6

Exploring the mechanism of C_4 photosynthetic differentiation through a unified comparative analysis of maize and rice leaf transcriptomes

(presented by Lin Wang <lwang@danforthcenter.org>)
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Maize and rice are the two most economically important grass crops and utilize distinct photosynthetic mechanisms to fix carbon. Rice, like most plant species, directly fixes carbon into two C_3 acids via the Calvin-Benson cycle. Maize, a semi-tropical plant, uses a two-step process with a C_4 acid intermediate that reduces photorespiration and affords higher water and nitrogen use efficiencies under hot arid conditions. We developed a mathematical model to compare transcriptomes from these two species along a unified leaf developmental gradient and define candidate cis-regulatory elements and transcription factors driving photosynthetic gene expression. The power of this comparative approach is illustrated by identification of an aerial suberin biosynthetic pathway for maize, a novel innovation associated with C4 photosynthesis. These resources will enable the elucidation and engineering of C_4 photosynthetic networks to improve the photosynthetic capacity of C_3 and C_4 grasses.

Funding acknowledgement: National Science Foundation (NSF)

SESSION 3 - QUANTITATIVE GENETICS
Chair: Milena Ouzunova Friday, March 15. 10:45 AM – 12:25 PM

T7

Variation of recombination landscape in European Flint and Dent maize

(presented by Eva Bauer <e.bauer@tum.de>)
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European hybrid maize breeding mainly employs two heterotic groups: (1) Northern European Flint inbreds which were developed from European Flint landraces, and (2) the Dent group which was mainly derived from early maturing North American Corn-Belt inbreds introduced to Europe in the middle of the 20th century. In the framework of the Plant-KBBE project “Cornfed” we have developed two panels of half-sib families for the European Dent and Flint pools for genome-wide association mapping and genomic prediction of agronomic traits. In each panel, one central line was crossed to genetically diverse founder lines from the same pool, with 11 and 12 populations for the Dent and Flint panels, respectively. In total, more than 2,200 doubled haploid lines were analysed. We constructed high-density linkage maps for each population using the MaizeSNP50 array. On average, around 12,000 SNPs were mapped in each population. The agreement among these maps and with the B73 reference sequence AGPv2 was high. Genetic maps from Flint crosses were on average 1,645 cM and significantly longer than maps from Dent crosses which were on average 1,353 cM. Taking the physical map of B73 AGPv2 as a reference, this translates also into differences in genome-wide recombination rates, with Flint having higher values than Dent (0.80 versus 0.66 cM/Mbp). No correlation between parental genome similarity and recombination rate was observed across 10 Mbp intervals. We characterized the recombination landscapes in the Dent and Flint panels and carried out a comparative analysis of chromosome-wide as well as local recombination frequencies between Flint and Dent pools and across individual populations. Recombination landscapes were analysed independently from the chromosome-wide recombination rates using normalized data. On chromosomes 2, 4, 5, and 6 highly significant differences between recombination landscapes were found between the Flint and Dent pools.

Funding acknowledgement: PLANT-KBBE Initiative Cornfed: ANR (France), MICINN (Spain), BMBF (Germany)

T8

From Many, One: Genetic Control of Prolificacy during Maize Domestication

(presented by David Wills <dwills@wisc.edu>)
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A reduction in number and an increase in size of inflorescences is a common aspect of plant domestication. When maize was domesticated from teosinte, the number and arrangement of ears changed dramatically. Teosinte has long lateral branches that bear multiple small ears at their nodes and tassels at their tips. Maize has much shorter lateral branches that are tipped by a single large ear with no additional ears at the branch nodes. To investigate the genetic basis of this difference in prolificacy or the number of ears on a plant, we performed a genome-wide QTL scan. A large effect QTL for prolificacy (*prol1.1*) was detected on the short arm of chromosome one in a location that has previously been shown to influence multiple domestication traits. We fine-mapped *prol1.1* to a 2.7 kb interval or "causative region" upstream of the grassy *tillers1* (*gt1*) gene, which encodes a class I homeodomain leucine zipper transcription factor. Tissue *in situ* hybridizations reveal that the maize allele of *prol1.1* is associated with a distinct pattern of *gt1* expression in the nodal plexus of developing lateral branches. The expression of *gt1* in the nodal plexus appears to suppress secondary ear development, resulting in the domesticated phenotype of a single ear per lateral branch. Population genetic analyses indicate positive selection on the maize allele of *prol1.1*, causing a partial sweep that fixed the maize allele throughout most of domesticated maize. This work helps clarify the underlying genetics of the changes in plant architecture that improved the harvestability of maize.

Funding acknowledgement: National Science Foundation (NSF)

T9

Identification of genetic factors conferring resistance to Western corn rootworm beetles, “The Billion Dollar Pest of Maize”

(presented by David Hessel <dhessel@iastate.edu>)
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One of the most damaging and economically important insect pests of maize is the Western corn rootworm (WCR). Despite widespread presence of WCR in the corn belt, U.S. corn has been protected by conventional means such as crop rotation, as well as by insecticidal transgenes. However, rotation-resistant WCR populations have been detected in several states, and reports of field-evolved resistance to *Bt* toxins are becoming increasingly frequent. Significant research efforts to improve the efficacy and epidemiological durability of insecticidal transgenes is ongoing, but there is also an increasing need to leverage native resistance (NR) mechanisms. Thus, a principle challenge is to identify and characterize the effects of beneficial natural alleles, both as substrate for breeding and mechanistic investigations.

Following three generations of selection imposed on a broad array of GEM accessions, we identified an exotic stiff-stalk accession, FS8(B)S016, as having strong potential for harboring NR alleles. In subsequent evaluations across seasons and locations, our FS8(B)S016 families outperformed all other accessions tested, and competed favorably with the transgenic check. We bred FS8(B)S016 with B86, an Iowa Stiff Stalk Synthetic line and subsequently derived F_2, BC_1, doubled-haploid, and PHG84 X doubled-haploid testcross analysis populations to enable genetic dissection of the observed resistance to larval feeding by corn rootworms. Our QTL analyses have consistently detected significant genetic effects that confer small to modest NR levels, which is meaningful in view of the economic scale and previous recalcitrance of this resistance trait. The success of this work shows that natural variation for resistance to WCR larval feeding is both heritable and experimentally tractable. We will discuss the importance of using doubled haploid lines, both in terms of the need for replicated trials and the value of evaluating these agronomically important alleles in both “inbred” and hybrid contexts.

Funding acknowledgement: USDA-NIFA, NSF-IGERT

T10

A Connected Set of Genes Associated with Programmed Cell Death Implicated in Controlling the Hypersensitive Response in Maize.

(presented by Bode Olukolu <baolukol@ncsu.edu>)
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Rp1-D21 is a maize auto-active resistance gene conferring a spontaneous hypersensitive defense response (HR) of variable severity depending on genetic background. We report an association mapping strategy based on the Mutant Assisted Gene Identification and Characterization (MAGIC) approach to identify naturally-occurring allelic variants associated with phenotypic variation in HR. Each member of a collection of 231 diverse inbred lines of maize constituting a high-resolution association mapping panel were crossed to a parental stock heterozygous for *Rp1-D21* and the segregating F_1 generation testcrosses were evaluated for phenotypes associated with lesion severity for two years at two locations. A genome-wide scan for associations with HR was conducted with 47,445 SNPs using a linear mixed model that controlled for spurious associations due to population structure. Since the ability to identify candidate genes and the resolution of association mapping are highly influenced by linkage disequilibrium (LD), we examined the extent of genome-wide LD. On average marker pairs separated by more than 10 kbp had an r^2 value of less than 0.1. Genomic regions surrounding SNPs significantly associated with HR traits were locally saturated with additional SNP markers in order to establish local LD structure and precisely identify candidate genes. Six significantly associated SNPs at five loci were detected. At each locus, the associated SNP was located within or immediately adjacent to candidate causative genes predicted to play significant roles in the control of programmed cell death and especially in ubiquitin pathway-related genes. The same strategy is currently been extended to the maize nested association mapping (NAM) population. Results from these analyses will also be presented.

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

T11

Genome-wide association study dissects the genetic architecture of quantitative traits in maize

(presented by Jianbing Yan <yjianbing@mail.hzau.edu.cn>)
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Association mapping through linkage disequilibrium (LD) analysis is a powerful tool for the dissection of complex agronomic traits and for the identification of alleles that can contribute to the enhancement of a target trait. With the developments of high throughput genotyping techniques and advanced statistical approaches as well as the assembling and characterization of multiple association mapping panels, maize has become the model crop for association analysis. The rapid development in genome-wide genotyping techniques promises to improve the power of association mapping and significantly refine our understanding of the genetic architecture of complex quantitative traits. We have build up a maize association mapping panel containing 500 diverse maize inbred lines which has been genotyped by using Maize 50K SNP chip and deep RNA sequencing technology. Totally, 1.06 million high quality maize SNPs were obtained and the genetic architecture of many complex quantitative traits was further extensively examined. Using oil concentration as an example, we identified 74 loci significantly associated with target traits which were further examined using eQTL mapping, linkage mapping, and co-expression analysis. More than half of the identified loci co-localized within mapped QTL intervals and one-third of the candidate genes were annotated as enzymes in oil metabolic pathway. The 26 loci associated with oil concentration could explain up to 83% of the phenotypic variation using a simple additive model. Results illuminate the process of oil synthesis in maize kernels, and may facilitate marker based breeding for oil quantity and quality. We will also discuss the potential use of this rich dataset for exploring other complex quantitative traits.
Partial of the mentioned results has been published in Nature Genetics (Li et al, Nature genetics, 2013, 45:43-50)

Funding acknowledgement: National Science Foundation of China, 863 project

T12

Phenotypic and transcriptional impacts of a specific copy number variation (CNV) in maize

(presented by Tao Zuo <taozuo@iastate.edu>)
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Copy number variation (CNV) has been revealed to be widespread in eukaryotic genomes and to play an important role in genomic evolution and environmental adaptation. However, the question of how CNV contributes to phenotypic diversity and how genes in the CNV region respond to copy number change in maize still remains unanswered. Here, a copy number variation allele, *p1-ww714*, isolated from *Ac/Ds* -induced sister chromatid transposition (Zhang and Peterson 1999; Zhang and Peterson 2005) was conducted to study the functional importance of a specific CNV in maize. The *p1-ww714* allele contains a 14.7 Mb inverted tandem duplication in chromosome 1S from 48.2 Mb to 62.9 Mb which represents about 10% of the total length of maize chromosome 1S. Our phenotypic data shows that the 14.7 Mb duplication in maize chromosome 1S has significant effects on plant height, flowering time, ear length and kernel number. Transcriptional data generated from RNA-seq and GeneChip (new Affymetrix Maize WT 100K array) shows that genes within the duplicated segment are clearly overrepresented among all of the differentially expressed genes detected. About 30% of genes in the duplicated region exhibit dosage-dependent expression, and the remaining 70% of genes are dosage compensated. Some genes outside the duplicated segment are differentially expressed and may represent the trans-effects of the duplicated genes. Statistical tests indicate that dosage-dependent genes are randomly distributed in the 14.7 Mb duplication and are not enriched in any functional category. Our results provide insight into the gene expression and phenotypic impacts of a recent maize CNV.

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

T13

Genome-wide Single Base Resolution Allele Specific DNA and Histone Methylation Profile in Maize Endosperm

(presented by Mei Zhang <zhangmei@cau.edu.cn>)
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Allele specific maps of epigenetic modifications are fundamental for understanding the regulation of genetic imprinting. Here we report genome-wide base resolution allele specific maps of DNA methylation and H3K27me3 in maize endosperm. Thousands of parental-of-origin dependent differentially methylated regions (pDMRs) were identified, which are uniformly maternally hypomethylated and paternally hypermethylated. We also identified more than 1,000 allele-specific H3K27me3 peaks, and they all preferentially presented in the maternal alleles. Correlation of allele specific DNA methylation, H3K27me3 profile and the expression of imprinted genes demonstrated that maternally expressed genes (MEGs) and paternally expressed genes (PEGs) are regulated by different mechanisms. The majority of PEGs are under regulation of both DNA methylation and H3K27me3, with their maternal alleles repressed by maternal specific DNA demethylation and subsequent H3K27me3. MEGs however are not directly contributed by H3K27me3, while about a quarter of MEGs are associated with maternal specific DNA demethylation. Our study provides important resources and patterns of regulation of imprinting in maize endosperm.

Funding acknowledgement: National Basic Research Program (973 program) (2009CB118400)

T14

Getting Hitched: retrotransposons, chromosome movement and centromere drive

(presented by Gernot Presting <gernot@hawaii.edu>)
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Maize centromeres contain centromere-specific retrotransposons (CR) and variable amounts of the tandem repeat CentC. The role of these repeats in centromere function has not yet been fully characterized, but recent *in vitro* evidence indicates that both repeats preferentially bind nucleosomes containing the centromeric histone H3 variant CENH3 over canonical nucleosomes, which is confirmed by yeast assays. CR elements target their insertion to the functional centromere regions of the host genome, and we recently discovered tandem repeats derived from extant CRM elements. Taken together, these data suggest that the tandem centromere repeats that commonly occur in centromeres may be derived from retrotransposons with characteristics similar to CR elements. The documented ability of CR elements to generate more active variants by recombination provides a mechanism by which they might adapt to changing requirements for centromere compatibility over evolutionary time.

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

SESSION 6 - BIOCHEMICAL & MOLECULAR GENETICS

Chair: Peter Balint-Kurti Saturday, March 16. 8:15 AM – 10:15 AM

T15

A genetic and biochemical basis for natural variation in maize aphid resistance

(presented by Georg Jander <gj32@cornell.edu>)
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Although there is considerable natural variation in maize resistance to insect herbivores, the underlying genetic basis of such variation has been identified only rarely. *Rhopalosiphum maidis* (corn leaf aphid) exhibits a 100-fold range in progeny production on the maize nested association mapping (NAM) population. This genetic variation allowed mapping of an aphid resistance quantitative trait locus (QTL) to a chromosomal interval with 31 genes. Analysis of maize defense metabolites showed that, whereas DIMBOA-Glc (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside) predominates in B73, the methylated form, HDMBOA-Glc (2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside), is more abundant in aphid-sensitive maize lines. Further genetic mapping co-localized QTL for high HDMBOA-Glc content and aphid sensitivity. Analysis of heterogeneous inbred families (HIFs) produced from a NAM line confirmed the defensive function of the QTL. This suggested that the underlying genetic basis for both traits would be a DIMBOA-Glc methyltransferase, an enzyme that was not encoded by any previously known genes. Cloning and *in vitro* enzyme assays confirmed that candidate genes in the QTL mapping interval encode methyltransferases that convert DIMBOA-Glc to HDMBOA-Glc. A coding-region transposon insertion, which inactivates one of the three predicted DIMBOA-Glc methyltransferases in B73 and certain other inbreds, is absent in the more aphid-sensitive maize lines. The observation of improved aphid growth on maize with relatively high HDMBOA-Glc content stands in marked contrast to previous research showing that defense-induced HDMBOA-Glc production provides caterpillar resistance. However, there is variation in the secondary defenses induced by benzoxazinones. In particular, we find less callose formation as an aphid defense response in maize lines with high HDMBOA-Glc. Thus, natural variation in DIMBOA-Glc methyltransferase provides a defensive tradeoff, with higher HDMBOA-Glc accumulation causing caterpillar resistance and aphid sensitivity. Interestingly, inbred lines with high HDMBOA-Glc in this study are all tropicals, suggesting targeted breeding for maize resistance to chewing herbivores at the expense of aphid sensitivity.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Defense Advanced Research Projects Agency (DARPA)

T16

The mapping, genetic analysis, and phenotypic characterization of *sugary enhancer1* (*se1*).

(presented by Karl Haro von Mogel <kmogel@wisc.edu>)
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sugary enhancer1 (*Se1*) is a naturally occurring allele that modifies endosperm composition in sugary (*su1*) maize, and is important for sweet corn quality. When in combination with *su1*, the recessive *se1* increases sugar content while maintaining high levels of water-soluble polysaccharide (WSP) in the endosperm. Using a unique genetic background that Mendelizes the *se1* locus in an isogenic background, we have genetically mapped the trait to the long arm of chromosome 2. The B73 reference *Se1* gene contains a predicted 522 bp ORF with a high (80%) GC content and no apparent introns. *Se1* is expressed in the endosperm and in developing leaf tissue. The recessive *se1* allele is the result of a 630 bp deletion that eliminates the ORF resulting in loss of normal *Se1* transcript and function. A 24 kb region surrounding the gene, and bounded by the adjacent gene models was sequenced, revealing differences between *Se1* and *se1* genotypes and confirming the absence of other potentially causative genes. *Se1* encodes a unique gene that is specific to monocots, and understanding its function in starch synthesis may lead to a deeper understanding of how different plants modulate starch metabolism.

Funding acknowledgement: United States Department of Agriculture (USDA), Grant no. CSREES/NRI 2007-55301-18179

T17

Defining the genetic and metabolic networks responsible for surface hydrocarbon production on maize silks

(presented by Marna Yandeau-Nelson <myn@iastate.edu>)
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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). In addition, the cuticle mitigates mechanical stresses imposed on silks encased within the husks. This cuticle is particularly abundant in hydrocarbons of 19-35 carbon atoms, however the genetic and metabolic networks responsible for production of these hydrocarbons 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.

We have demonstrated that the complex arrays of surface hydrocarbons vary dramatically among maize genotypes, during silk development and according to environmental conditions, suggesting complex regulation of this metabolic network. For fine-scale elucidation of the genetic network, we have executed a full-scale metabolite-QTL mapping experiment using 660 IBM mapping lines, which possess considerable variation in the surface lipid metabolome. QTL analysis of 56 hydrocarbon traits identified a total of 212 genetic loci that modulate the composition of the hydrocarbon metabolome. Notably, we detect genetic regulators for the accumulation of individual metabolites as well as for classes of metabolites (e.g. all alkanes of odd-numbered chain length). Positional resolution is very high in this experiment, with many QTL regions harboring only tens of genes. We have cross-referenced these gene lists with gene annotations and RNAseq results from two stages of silk development, which differ ~3-fold in hydrocarbon accumulation (i.e. emerged vs. encased silks). We will present several examples of candidate gene hypotheses that are supported by multiple lines of evidence and we will discuss how these cases are shaping our network models of hydrocarbon biosynthesis and regulation.

Funding acknowledgement: National Science Foundation (NSF), United States

T18

Deep mRNA-sequencing of *rough endosperm3* seedlings reveals altered splice site usage due to reduced function of splicing factor URP

(presented by Christy Gault <cgault@ufl.edu>)
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The *rough endosperm3* (*rgh3*) mutant demonstrates that alternative splicing plays a critical role in maize endosperm and seedling development. The *rgh3* mutation is a hypomorphic allele of the U2AF^{35}-related protein (ZmURP), whose human ortholog is a splicing factor that is associated with the minor and major spliceosomes. Mutant *rgh3* kernels are reduced in grain-fill with a rough surface, and less than 50% of mutant seeds germinate. During seed development, *rgh3* endosperm cells show developmental delays and a failure to differentiate specialized cell types. Mutant seedlings have adherent leaves, stunted growth, and die 15-18 days after planting. To identify global mRNA splicing defects in *rgh3*, we sequenced the transcriptomes of *rgh3* and wild-type seedlings. Root and shoot cDNA libraries were constructed from three seedlings of each genotype. The resulting twelve libraries were multiplexed and sequenced on one lane of the HiSeq 2000 platform, producing 149 million reads that mapped to more than 35,000 genes. Bioinformatic analysis of the RNA-seq data identified forty-five genes that were predicted to have altered splicing in *rgh3* seedlings. A subset of these predictions was validated by RT-PCR and sequencing of the altered splice variants. Genes with altered splicing in *rgh3* tend to have U12-type introns and function in cytoskeletal organization, vesicle trafficking, and cell cycle regulation. These data suggest the hypomorphic *rgh3* protein preferentially affects minor spliceosome activity

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

T19

Lipid-mediated signaling between maize and pathogens is required for disease development or defense

(presented by Eli Borrego <eli.borrego@gmail.com>)
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Intimate interactions between plants and other organisms are orchestrated by lipid signals produced by the pair. A class of oxygenated lipids, termed oxylipins, has recently taken center-stage for their roles in defense and disease development during fungal infections. These metabolites are produced through dioxygenases in both plants and fungi where they possess potent endogenous signaling activity for diverse processes. In plants, the best understood oxylipins are undeniably the jasmonates and green leaf volatiles generated by the lipoxygenase (LOX) pathway, which regulate defense, development, and inter-organismal communication. Psi producing oxygenases (Ppo) are the major contributors for oxylipin biosynthesis in fungi, and here they modulate sporulation and secondary metabolism. Remarkably, oxylipins from both kingdoms are structurally and biochemically similar, prompting an exciting hypothesis; during plant-fungal interactions such as during fungal infections, oxylipin signals are reciprocally exchanged between host and parasite. G-protein coupled receptors (GPRCs) are implicated as the means of perception of these oxylipin-mediated signals. Employing a reverse genetics approach in the maize-Aspergillus pathosystem, our laboratory has found that the unique bouquet of oxylipins generated by both host and pathogen might indeed determine the outcome of the interaction and the ability of fungi to successfully infect, colonize, and reproduce. This knowledge will spearhead understanding molecular mechanism behind oxylipin-mediated signal exchange during plant-fungal interactions and may allow development of novel environmentally friendly disease resistance strategies.

Funding acknowledgement: National Science Foundation (NSF)

T20

Map-based cloning and characterization of a maize yellow seedling mutant and its modifier

(presented by Angi Xing <anqi.xing1984@gmail.com>)
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A yellow seedling (ys) mutant was identified from a B73 EMS mutagenized population. Homozygous mutant plants exhibit a pale-yellow leaf phenotype within the first 10 days after emergence, and the phenotype recovers 2 weeks after emergence. Microscopic analyses demonstrated that chloroplast development in ys is impaired, with smaller chloroplasts and lower chlorophyll content in the mutant plants. Map-based cloning with a B73ys/Mo17 BC1 population identified the maize clpP1 gene on chromosome 9, which encodes one subunit of the chloroplast Clp protease complex, as the candidate gene for ys. A 141bp insertion at one exon-intron junction was identified in ys, which affects the splicing of the clpP1 pre-mRNA. The ys candidate gene was validated by a Mu-insertional allele of clpP1 and by allelism test. When ys was introgressed into PH09B (a Pioneer inbred line), the mutant phenotype lasted much longer in greenhouse and the mutant plants failed to grow to maturity in the field, implying the presence of a modifier for the ys gene. A major ys modifier was identified on chromosome 1. Map-based cloning with a B73ys/PH09Bys F2 population identified a paralogous clpP1 gene as the candidate for the ys modifier. A Mu-insertional allele of the paralogous clpP1 has been identified and allelism test is under way. No major sequence variations were observed in the chr.1 clpP1 gene between B73 and PH09B. However, the expression of the chr.1 clpP1 gene is induced in B73 or Mo17 background when the chr.9 clpP1 gene is mutated (ys), while similar expression induction is not observed in PH09B. We hypothesize that elevated expression of the paralogous clpP1 gene is responsible for the recovery of the ys mutant phenotype in B73 or Mo17. Lack of induction of the paralogous clpP1 gene results in the delayed recovery of the ys phenotype in PH09B.

Funding acknowledgement: China Scholarship Council, DuPont Agricultural Biotechnology

SESSION 7 - CELL & DEVELOPMENTAL GENETICS
Chair: Mark Settles Saturday, March 16. 10:45 AM – 12:25 PM

T21
Origin and Genetics of Kranz Anatomy and C4 Anatomical Specialization

(presented by Thomas Slewinski <tls98@cornell.edu>)
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A worldwide effort is underway to engineer the C4 photosynthetic pathway in rice and other C3 crops. It has been estimated that success in this ambitious venture could boost yield by 50%. The major obstacle at present is that the genetic basis for development of specialized C4 leaf anatomy is a mystery. The key to C4 specialization is that two independent carbon fixation reactions must be compartmentalized for C4 photosynthesis to work. In maize, and many other C4 plants, compartmentation is accomplished by a unique leaf architecture, known as Kranz anatomy, in which bundle sheath (BS) and mesophyll cells are arranged in concentric rings around the veins. Investigations into anatomy and development of this tissue has lead to a new hypothesis that would explain the repeated convergent evolution of Kranz-type C4 photosynthesis in plants. In brief, Kranz anatomy and C4 physiology is a result of the endodermal tissue, usually only present in roots and stems, projecting into the leaf blade. Using reverse genetics to identify mutants in maize, we were able to show the Scarecrow gene, which regulates endodermal identity in roots, also regulates Kranz anatomy. Mutations in Scarecrow result in proliferation of BS cells, abnormal differentiation of BS cells and BS chloroplasts, loss of minor veins, and reduction of vein density, which are all key features of C4 structure. This finding, that C4 may arise from an established and highly conserved repertoire of signals that governs endodermal differentiation in roots and stems, casts a more optimistic light on the task of engineering Kranz-type C4 into C3 crops.

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

T22
Transport of boron and water by the *tassel-less1* aquaporin is critical for vegetative and reproductive development in maize

(presented by Amanda Durbak <durbaka@missouri.edu>)
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The essential plant micronutrient boron (B) is required for normal growth and development. B deficient conditions, particularly in grasses, result in reproductive defects that lead to significant reductions in yield. We have identified the *tassel-less1* (*tls1*) mutant that has severe defects in inflorescence development, including absent or sparse tassels with reduced main spike and aborted or ball-shaped ears. Positional cloning revealed that *tls1* encodes a major intrinsic protein in the aquaporin family co-orthologous to the Arabidopsis B influx transporters *AtNIP5;1* and *AtNIP6;1*. Transport assays in *Xenopus* oocytes indicate that the TLS1 protein transports both B and water. Genetic analysis between mutations in *tls1* and *rotten ear* (*rte*), the proposed B efflux transporter, revealed that *tls1;rte* double mutants have defects similar to strong *tls1* single mutants. Additionally, plants heterozygous for both *tls1* and *rte* show defects in inflorescence development including reduced branch and spikelet number in the tassel, reduced kernel row number and ear length, and sterility. These dosage results suggest that the defects observed in *tls1* mutants are due to B deficiency in the shoot. As very little is known about the functions of B in plants, besides its role in cross-linking cell wall sugars, we are using the *tls1* mutant as a tool to elucidate the role of B in plant growth and development.

Funding acknowledgement: National Science Foundation (NSF)

T23

Control of Maize Shoot Architecture by Brassinosteroid (BR) signaling

(presented by Gokhan Kir <gkir@iastate.edu>)
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Brassinosteroids (BRs) are phytohormones that have important roles in plant development such as sex determination, internode elongation, and leaf development. Research on Arabidopsis and rice revealed a general conservation in the key components of BR signaling. However, BR biosynthesis and signaling in maize are not well characterized. Understanding BR's role in maize might help manipulate this crop for production of biofuels, biomass, and grain yield. To understand some of the signaling components of BRs in maize, we took a transgenic approach to generate maize plants altered for different BR related gene functions. For this purpose, *bri1*, which encodes the BR receptor , was targeted by an RNAi approach. BLAST searches identified 5 BRI1 homologs in maize, with the highest scores on chromosomes 8 and 5. A BRI1RNAi construct was designed using the extracellular domain of the chromosome 8 copy, which we designated BRI1. The nucleotide sequence used in the construct is expected to target both the chromosome 8 and 5 transcripts and endogenous gene expression analyses confirm that both genes are downregulated in *bri1RNAi* transgenic lines. Other BRI1 homologs also show decreased expression in transgenic lines. Analysis of BR marker genes showed that *brd1* and *cpd* genes are upregulated in transgenic lines, consistent with disrupted BR signaling.BRI1RNAi plants showed dwarf stature due to shortened internodes, with later internodes more strongly shortened compared to early ones. Analysis of internode epidermal cells suggests that internode shortening is due to decreased cell size. Leaves are dark green, upright, and twisted, also showing altered auricle morphology. Expression of BES1-YFP, a BR reporter, suggests that BR signaling is active in the auricle region of developing leaves. In addition, we targeted BIN2 by RNAi suppression. BIN2 is a negative regulator of the BR signal transduction system and suppression is expected to cause upregulation of BR signaling. Tassel branch internodes and leaf blades were elongated, but unexpectedly, stem internodes were shortened.

Funding acknowledgement: Plant Sciences Institute at Iowa State University

T24

Warty2 encodes a putative receptor-like Tyr kinase that contributes to maize leaf blade cell expansion.

(presented by Anding Luo <aluo@uwyo.edu >)
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The maize leaf epidermis is useful for studying cell division and expansion because blade cells are organized in regular linear arrays. Mutations that alter the normal cell pattern can be detected by screening mutant populations for overly expanded or disorderly cells. A recessive EMS mutant (courtesy of Tom Brutnell) was designated *warty2* (*wty2*) due to its superficial similarity to another cell pattern mutant, *wty1*. The *wty2* mutant shows wrinkled and recurved leaf blades due to excessive division and expansion of epidermal cells. These expanded epidermal cells are bulliform-like in appearance based on histochemical staining and cellular structure. *Wty2* was mapped to chromosome 3L based on segregation of SNPs in a Mo17/B73 segregating population. Subsequently, the gene was mapped within an interval of about 32 kb. DNA sequence analysis uncovered a point mutation in a candidate gene. The gene identity was further confirmed by three additional alleles: one by EMS mutagenesis and two by reverse screening of UniformMu lines. In addition, the transgenic lines of three constructs also complement the mutation, including a complementation construct, an over-expression and a WTY2-YFP reporter line. WTY2-YFP localizes to the plasma membrane in young dividing and expanding leaf tissue and appears polarized to the membrane in the new cell wall consistent with its proposed function during early division and expansion. *Wty2* encodes a putative receptor-like Tyr kinase with an inactive kinase domain, implying a potential function in signal transduction. The role and function of this protein during leaf patterning are being explored.

Funding acknowledgement: NSF IOS #1027445

T25

FASCIATED EAR 4 encodes a bZIP transcription factor required for maize meristem size homeostasis

(presented by Michael Pautler <pautler@cshl.edu>)
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Plant architecture is dictated by the precise control of meristematic activity. An imbalance in positive or negative maintenance signals can result in a fasciated meristem phenotype. *fea4* is a semi-dwarfed mutant with fasciated ears and tassel due to greatly enlarged vegetative and inflorescence meristems. We mapped *fea4* to a 2.7 Mbp region of chromosome 6 containing approximately 30 genes. A bZIP transcription factor in this interval contained an EMS-induced early stop codon in the reference allele. A second allele, originally identified as a modifier of *ramosa2*, also contained a premature stop codon, confirming the identity of the gene. Subsequently, we isolated additional alleles from EMS and transposon mutagenized stocks. Phylogenetic analysis suggests that *fea4* is orthologous to the Arabidopsis gene *PERIANTHIA*, which has an analogous, but less severe, loss-of-function phenotype. We carried out in situ hybridization to determine the expression pattern of *fea4* throughout development. During the vegetative phase, *fea4* is expressed specifically in the peripheral zone of the SAM and in the vasculature of immature leaves. *fea4* is conspicuously excluded from the stem cell niche at the tip of the SAM and the incipient leaf primordium (P0), but strongly enriched in a domain beneath the P0. Following transition to reproductive fate, *fea4* is expressed throughout the entire inflorescence meristem, and also throughout the spikelet-pair, spikelet, and floral meristems. Expression of a YFP-FEA4 translational fusion protein under control of the native promoter recapitulated the pattern of expression observed by in situ hybridization. We have profiled transcriptional changes in 1mm ears of mutants relative to wild-type by RNA-seq, and are beginning to explore potential targets. Genetic analysis suggests that fea4 functions in parallel to the *fea2-td1 (CLAVATA)* pathway, suggesting that it defines a novel pathway in meristem size regulation.

Funding acknowledgement: National Science Foundation (NSF), DuPont Crop Genetics; NSERC

SESSION 9 - NEW RESOURCES & APPROACHES TO MAIZE RESEARCH
Chair: John Fowler Sunday, March 17. 8:30 AM – 9:50 AM

T26
Reconstruction of Protein Networks from an Atlas of Maize Seed Proteotypes

(presented by Justin Walley <jwalley@ucsd.edu>)
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Genome-wide, quantitative proteomics makes possible the creation of a protein atlas that catalogs where, when, and how much of a given protein is present. Using quantitative mass spectrometry we mapped an atlas of proteotypes (i.e. proteomic state) comprising 14,165 proteins and 18,405 phosphopeptides (from 4,511 proteins), quantified across eight tissues of the developing maize seed. Comparisons between proteotypes revealed the quantitative contribution of specific proteins to endosperm starch and embryo oil biosynthesis. Also revealed were sites of dynamic phosphorylation that may regulate the associated transporters and biosynthetic enzymes. Reconstruction of signaling networks from the proteotypes related proteins and phosphoproteins to biological processes acting during seed development. The networks identified known and predicted novel substrates of protein kinases. Finally, correlation between protein and mRNA levels was poor and many of the most abundant proteins were not associated with mRNAs.

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

T27
A sequence-indexed reverse genetics resource for maize

(presented by Yubin Li <yubin@waksman.rutgers.edu>)
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User-friendly sequence-indexed reverse genetics resources are vital to efficiently exploit the maize genome sequence in the post-genomic era. Our NSF-PGRP-funded project is generating and sequence-indexing a collection of *Ac* and *Ds* insertions using a cost-effective method that takes advantage of next-generation sequencing technologies. Specifically, we are: (1) Sequence-indexing an existing collection of over 1000 *Ac* transposants from *wx-m7(Ac)*; (2) Assembling a set of 120 roughly equidistant *Ds** launching platforms carrying a *GFP* marker that allow simple visual selection of element transposition from any region of the genome and, thus, enable researchers to generate regional gene knock-out collections; (3) Sequence-indexing several thousand *Ds** insertion sites from model platforms by high throughput sequencing of 3-dimensional DNA pools with the 5500 SOLiD™ System; and (4) Updating all relevant information to our web-searchable database of insertion site sequences (http://acdsinsertions.org) cross-referenced to stocks available from the Maize Genetics Co-op.

The following is a summary of our current progress. (1) Taking advantage of high throughput sequencing technologies, we have mapped over 300 *tac* (*trAc*) or *tds* (*trDs*) sites to the maize genome. (2) Using a *C1* (colored seed) marker interrupted by a *GFP*-tagged *Ds** element, more than 200 *c1-m* transgenic lines with *Ds** transposition activity have been generated by *Agrobacterium* transformation and more than half of them have been mapped to the reference B73 genome. (3) More than 10,000 C' revertants bearing a *trDs** have been selected from lines with a high reversion frequency. In a test of 5,000 *C' GFP* (purple, green fluorescent) selections, >90% were heritable, showing that the system is extremely efficient for recovering *Ds** transposition. More than 1000 *trDs** target sites have already been mapped to the reference genome using next-generation DNA sequencing technology and 1000 others are currently being mapped. (4) All the lines generated in this project are listed in our web-searchable database and will be sent to the Maize Genetics Stock Center for distribution.

Funding acknowledgement: National Science Foundation (NSF)

T28

The Relative Contribution of Genic and Intergenic Polymorphisms to Natural Phenotypic Variation in Maize

(presented by Jason Wallace <jason.wallace@cornell.edu>)
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Genome-wide association studies are increasingly finding that changes in gene regulation are at least as important for natural variation as changes in protein sequence. We performed a large-scale, systematic evaluation of the relative contribution of structural (genic) and regulatory (intergenic) variations using >55 million polymorphisms from Maize Hapmap2. Our analysis consists of genome-wide associations for >40 phenotypes in the maize Nested Association Mapping population, using both single-nucleotide polymorphisms (SNPs) and read-depth variants (RDVs, a measure of copy-number variation) as explanatory variables. Genes with GWAS hits tend to be more highly expressed than those without, and most significant SNP hits occur near genes but outside of the actual transcript. RDVs are significantly enriched relative to SNPs for association and become more significant as they are binned at smaller scales, implying that small insertion/deletion mutations may drive a large portion of phenotypic variation. One important unknown is how far these patterns extend to variation that we currently lack the power to detect, such as rare alleles or regions not present in the reference genome. Identifying these variants and annotating the functional elements they affect will be a major challenge and opportunity for maize research.

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

T29

MaizeGDB: everything old is new again!

(presented by Carson Andorf <carson.andorf@usda.ars.gov>)
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The focus of genetic, genomic, and breeding research evolves over time, making it necessary to continually redefine the paradigm for data access and data analysis tools. Here we report the reinvention of MaizeGDB, the maize genetics and genomics database, to meet maize researchers' ever changing needs. New, emerging, and prevailing areas of research that guided the reinvention of MaizeGDB include the availability of a well-sequenced reference genome and resequencing information from literally thousands of diverse inbred lines and populations, as well as the emergence of computational tools that enable the execution of, e.g., detailed functional genomics analyses before ever setting foot in the wet lab or research plot. The overall goal of the 2-year MaizeGDB redesign has been to expand 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 and interaction tools) into the MaizeGDB resource. A key component to the redesign has been community involvement. Several community members have volunteered their time and perspectives as beta-testers for the new site and continue to provide valuable insight. In addition the community at large has offered perspectives via email, website feedback, and personal interactions. Here we provide an overview of the new website, updates on new and forthcoming data and data types, and describe the stages of release planned for the new site. To try out the new website now, visit us at http://alpha.maizegdb.org.

Funding acknowledgement: United States Department of Agriculture (USDA)

T30

Live 3D imaging reveals the effects of long-term recurrent selection for yield traits on maize root system architecture

(presented by Christopher Topp <chris.topp@duke.edu>)
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Changes to subterranean morphologies associated with selection for yield traits in crops are virtually unknown despite the importance of roots to plant physiology and environmental interactions. We used a previously developed 3D root imaging and analysis platform to investigate the changes in maize root architecture that occurred during two very different selection schemes: seed protein content, and grain yield at high planting density. The Illinois High and Low Protein lines have been recurrently selected for seed protein content for over 100 years, during which time the IHP lines have gained an increased nitrogen uptake capacity, whereas the ILP lines have become impaired in nitrogen uptake. We generated 3D root reconstructions of IHP and ILP lines, and quantified a number of significant architectural differences between IHP and ILP, including a striking lateral root phenotype indicative of enhanced nitrogen scavenging capacity in IHP. Using a similar methodology, we phenotyped hybrid Iowa Stiff Stalk Synthetic (BSSS) X Corn Borer (BSCB) lines that had been recurrently selected for grain yield at high planting density (cycle 17), or were unimproved for this trait (cycle 0). Cycle 0 hybrid root systems were significantly larger, but this biomass was allocated into a relatively wider, shallower, and more branched architecture compared to cycle 17. We also quantified responses to neighboring roots in these lines by growing two plants together in the same pot. Remarkably, the architecture of cycle 17 lines remained virtually unchanged, whereas cycle 0 lines became significantly smaller, shallower and less branched when challenged by a neighboring root system. Thus density-adapted maize lines appear to have a slimmer, deeper soil profile, as well as a dampened architectural response to intraspecific root competition. We also discuss an automated imaging and analysis platform that will measure the growth of individual roots from hourly 3D time-series over several weeks.

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

T31

VirtualMaize: A Software platform for translational Systems Biology research in Crops.

(presented by Manpreet Katari <mkatari@nyu.edu>)
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To enable Systems Biology studies across plant species, we have developed and expanded the VirtualPlant software platform to include Maize and Rice and Arabidopsis genomes. VirtualPlant (www.virtualplant.org), originally developed for an NSF Arabidopsis 2010 Grant, includes tools for data analysis, integration and visualization such as the Arabidopsis MultiNetwork interaction database, as well as novel data visualization tools including BioMaps and Sungear. VirtualPlant enables seamless integration of data and tools into a single software platform by virtue of its unique "Gene Cart", which enables researchers to store results, enabling iterative cycles of analysis, a highlight of Systems Biology. Additionally, VirtualPlant's web-based user-friendly GUI enables plant biologists to analyze their own genomic data, enabling them to uncover and infer biological insights.

VirtualPlant has played an integral part in enabling Plant Systems Biology research in Arabidopsis in prominent plant labs around the world. Our recent expansion of VirtualPlant to include important crops such as Maize and Rice, facilitates the comparisons of crop networks to Arabidopsis, hence enabling translational research. For example, VirtualPlant allows researchers to predict how an interacting network of genes/products in crop genomes will react as a system in response to an environmental change or genetic modifications, and predict genes in the crop to target for manipulation.

Here, we present a case study of how Virtual Maize can be used to enable hypothesis generation regarding Nitrogen-responsive gene networks in Maize, by transferring "network knowledge" of gene interactions (e.g. protein-protein, protein-DNA, etc) from Arabidopsis. Currently, VirtualPlant is cross-operational across Arabidopsis, Maize and Rice enabling translational network analysis. Since we have semi-automated the induction of new species from Phytozome (www.phytozome.net), additional crop genomes including Soy and Sorghum will be inducted into VirtualPlant in the near future.

Katari etal. VirtualPlant: a software platform to support systems biology research. Plant Physiol, 2010. 152(2): p. 500-515.

Funding acknowledgement: National Science Foundation (NSF)

T32

Growth Dyn-omics: studying the dynamics of transcriptomics and interactomics within the growth zone of the maize leaf

(presented by Hilde Nelissen <hilde.nelissen@psb.vib-ugent.be>)
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The maize leaf offers an excellent experimental system to study growth, due to the linear organization of cell division and expansion along its longitudinal axis: active cell divisions occur at the base of the leaf, and as the distance from the base increases cells will cease division and start expanding until they reach their mature cell size.

Recently, we found that bioactive gibberellins (GAs) peaked near the transition between the cell division and cell elongation zone, and that the balance between GA biosynthesis and degradation determines the position of the transition zone (TZ). We showed the functional importance of TZ for organ size since boosting GA biosynthesis in a *GA20-oxidase* overexpressing line and blocking GA production in the *dwarf3* biosynthetic mutant resulted in a shift of the TZ and thus in the number of dividing cells, resulting in larger and smaller leaves, respectively (Nelissen *et al*., 2012).

Besides genetic perturbations, we found that a mild drought treatment reduces the growth of the maize leaf by shifting TZ more basally, resulting in fewer dividing cells, stressing once more the importance of TZ in determining leaf size. In order to gain more detailed insights in the dynamic changes in the molecular processes that govern the position of TZ, we fine-sample throughout the growth zone for different '-omics' technologies. The presented data will show how specific transcriptional changes can be observed within the growth zone of the maize leaf. By applying tandem affinity purification of tagged proteins in maize, we visualize changes in protein complex composition along the transition zone of a maize leaf.

Taken together, the size and organization of the maize leaf growth zone allow the construction of a detailed map of the dynamics of molecular processes within a growing organ.

Poster Abstracts

P1

A Comparison of De Novo Genome Assemblers for Maize

(submitted by Hung-Ying Lin <hungying@iastate.edu>)
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The next-generation sequencing (NGS) revolution has generated unprecedented amounts of sequence data that can be used for genome assembly. Various genome assembly software packages are available but the performances of various assemblers are difficult to compare because results vary depending on read length, read quality, and features of the target genome, such as size and complexity. Here we used the ART Illumina reads simulator (version: grapewine) to generate sequence reads at different read depths (20x, 40x, 60x and 80x) using 5 fully sequenced Mo17 BACs. Simulated data sets were assembled with various assemblers using a multiple parameters. An assembly quality control pipeline was established to calculate the contig numbers, N50, mismatches, InDels, genome coverage, and memory use for each assembler. The best assembly output from series trials using different parameters was selected to represent the performance of a given assembler. This research is the first step in a larger effort that will guide the selection of tools for the de novo assembly of maize genomes.

P2

A Genome-Wide Analysis of the Expression of Alleles Containing Premature Stop Codons

(submitted by Alina Ott <aott@iastate.edu>)
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At many loci, mutant alleles that contain premature termination codons (PTCs) exhibit reduced levels of transcript accumulation as compared to non-mutant alleles. This reduction is a consequence of Nonsense Mediated Decay (NMD), a widely studied mechanism that targets for degradation PTC-containing RNA transcripts. We recently characterized two EMS-induced mutant alleles of the maize gl13 gene (Li et al. personal communication) that contain PTCs and that accumulate higher levels of gl13 transcript than a non-mutant control. Other similar counter-intuitive examples have been reported (e.g., Makarevitch et al., 2012, PLOS ONE). This motivated us to conduct a genome-wide scan of maize for PTC-containing alleles and determine the effects of these PTCs on transcript accumulation. Using deep RNA-seq data from multiple tissues of the 26 NAM founders plus B73 and Mo17, we scanned each FGS gene in each genotype for PTCs relative to canonical transcripts. Numerous PTCs were identified. Next, for every gene for which PTC-containing alleles were identified we compared transcript accumulation levels of PTC-containing and non-PTC alleles. Many genes were identified for which PTC-containing alleles exhibited higher transcript accumulation than non-PTC alleles. The potential roles of the positions and context sequences of PTCs, and chromosomal positions, epigenetic states and functional annotations of affected genes on transcript accumulation may provide novel insight into mechanisms that regulate gene expression.

Funding acknowledgement: National Science Foundation (NSF)

P3

A novel approach for subset selection of SNP markers for cost-effective implementation of genomic selection

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Molecular markers covering the whole genome have been successfully used for prediction of genetic merit of selection candidates, the so-called genomic selection. However, genotyping many individuals with high-density SNP marker panels may not be feasible in commercial breeding programs. In these circumstances an alternative is the selection of a subset of informative markers for generating a cost-effective SNP panel. The goal of this work was the evaluation of a novel approach for such a task, which involves two steps: selecting and fitting. The selecting step consisted of grouping the p available SNPs such that linkage disequilibrium levels are higher between SNPs within groups and lower between SNPs from different groups. Then, within each group a backward elimination approach based on least squares regression was used to exclude non-significant SNPs, based on an arbitrary significance level. Lastly, all SNPs selected from each group were combined, forming a final group with $q < p$ SNPs, and a Bayesian Lasso (BLasso) procedure was used to fit the final model. The procedure was applied to a dataset on maize containing 284 genotypes and 1148 markers for flowering traits, and 264 genotypes and 1135 markers for yield trait. We performed a 10-fold cross-validation to compare the predictive ability of the proposed approach against other commonly used methods: i) selection of SNPs equally spaced and model fit using ridge regression (RR-Blup); ii) selection of SNPs with larger estimated effects and model fit using BLasso; and iii) selection of SNPs with larger estimated effects and model fit using RR-Blup. Also, we compared the accuracy of the prediction of this new approach against the accuracy from the complete model (p SNPs) using the BLasso. Partial results show that our new approach performs better in many cases and allows for an optimized reduction on the final number of SNPs.

Funding acknowledgement: Coordination for the Improvement of Higher Level - or education - personnel (CAPES), Minas Gerais State Research Foundation (FAPEMIG)

P4

A panoramic view of long non-coding RNAs, their inheritance pattern and genetic mapping in maize

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Long noncoding RNAs (lncRNAs) are transcripts that are 200 bp or longer, do not encode proteins, and play an important role in eukaryotic gene regulation. However, the number, characteristic, regulatory roles, and inheritance pattern of lncRNAs in maize are still largely unknown. By exploiting available public ESTs, maize whole genome sequence annotation and RNA-seq datasets from 30 different B73 experiments, we identified thousands of lncRNA candidates, which indicates that a substantial number of transcript isoforms in maize are non-protein coding. Of these lncRNAs, approximately 2/3 are predicted to be the precursors of siRNAs, miRNAs and shRNAs, while the reminder tend to be intact lncRNAs. The average transcript length of the maize lncRNAs is 443 bp and they contain fewer exons than average (~1.3 exons for lncRNAs versus 3.5 for the Filtered Gene Set). We also noticed that the degree of sequence conservation of these maize lncRNAs to Arabidopsis, Rice and Sorghum is much lower than predicated maize protein-coding genes, even significantly lower than that of intron sequences and intergenic regions. Moreover, we explored the regulation of these lncRNAs in 13 distinct tissues, 18 inbred lines, and 105 IBM RILs of maize. More than 80% are expressed in a tissue-, inbred- or RIL-specific manner and tend to correlate ($P < 0.05$) with the expression of neighboring transcripts compared with protein-coding transcripts. Intriguingly, the inheritance pattern of lncRNAs in 105 IBM RILs may exhibit transgressive segregation, and lncRNAs in maize are less affected by cis- rather than trans-genetic factors. Our results provide a unique annotation resource of the maize genome, exhibit a panoramic view of lncRNAs and explore the inheritance principles of lncRNAs in maize.

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P5

Advancing complex phenotype analyses through machine vision and computation

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Machine vision utilizes information contained in an image or other optoelectronic signal, such as reflectance spectroscopy, to collect quantitative measures of phenotypes. We are integrating multiple machine vision platforms to study seed and seedling phenotypes focusing on the interrelationships of maize kernel traits with seedling growth traits. We have developed a semi-automated pipeline to collect kernel weight, near infrared reflectance (NIR) kernel spectra ranging from 910 nm to 1690 nm, 40 image features which describe kernel color and 3D shape, and 3rd-order feature tensor which characterizes the dynamics of seedling root growth. To preserve the highest quality in the phenotypic relationships, each kernel in the pipeline is given a unique identifier, which follows it through each phenotypic phase, from Florida to Wisconsin. To date, over 45,000 kernels have been run through the NIR spectroscopy and 3D-imaging platforms, and over 10,000 kernels have been germinated, gravitropically stimulated and imaged. Using computational approaches these raw data sets are transcribed into an integrated model for kernel chemical composition, kernel morphology and seedling growth. Raw, extracted and predicted data produced at separate locations are housed in a centralized SQL database, which allows for integrated modeling and analysis. Genotype to phenotype relationships are being explored via statistical genomics as well as phenotype-to-phenotype relationships via computational modeling.

Funding acknowledgement: National Science Foundation (NSF)

P6

Allelic horsepower: An outstanding result from examinations of qTeller-maize two-gene scatterplots

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As of January 27, 2013, the qTeller (qT) database contained 36 RNA-seq maize datasets deposited in the NCBI Sequence Read Archive by eight different research groups. This poster plots the FPKM values obtained by quantifying these diverse reads using a common RNA-seq analysis pipeline (Schnable and Freeling, 2013) for maize homeologous pairs. At the time of duplication these genes contained identical complements of regulatory sequence. Our figure comprises 36 FPKM: FPKM dots on the scatterplot visualized in the qT-maize web application: http://qteller.com/qteller3/scatter_plot.php?name1=GRMZM2G057973&name2=GRMZM2G004140&xmax=100&ymax=100. The pattern is a band of dots spread in a straight-line relationship, with slope 3.3. That means that one of these duplicates is approximately 3.3-fold more expressed in each of these 36 different RNA-seq experiments, even though these genes certainly specify RNA levels 0.5-25 FPMK for one gene and 0.5-82 FPMM for the other and report on expression in a diverse set of organs/cells from plants grown at different times, under different conditions, by different labs. This unequal linear relationship was the pattern most commonly observed when comparing homeologs.. These data appear to indicate that the developmental/physiological pattern of RNA expression is regulated separately from the intrinsic quantity of RNA expression horsepower. See Freeling and coworkers (2012) for a possible mechanism, one that borrows greatly from the Brandon Gaut lab.
We hope qTeller-Maize serves us all well as RNA-seq data accumulate. qT is more useful when maize datasets are more comparable. We recommend using B73 provided by the North Central Regional PI Station, Ames, Iowa, USA, PI 550473, since 1972, an accession most similar to refgen.

Schnable J, Freeling M. 2013… PLoS-One submitted.
Freeling M. et al 2012. Fractionation mutagenesis …Current Opinion in Plant Biology 15(2): 131-139.

qT thanks all who have contributed to our maize RNA-seq community resource, and our poster will cite them properly.

Funding acknowledgement: National Science Foundation (NSF)

P7

Coevolution of centromeres and foundation kinetochore proteins in the genus *Zea* and the broader Poaceae

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Expansion of repeat arrays in centromeres could enable a chromosome to more extensively recruit kinetochore proteins and spindle fibers. These chromosomes would then migrate more quickly during meiosis causing them to be preferentially incorporated into the female gamete. This phenomenon, known as centromere drive, can have profound evolutionary implications and may explain rapid differentiation previously observed in centromeres. We have resequenced DNA-binding domains of the foundation kinetochore proteins CenH3 and CenPC in samples of maize, its wild relatives, and the broader Poaceae. In addition, we have generated full-genome, short-read data from these same individuals in order to estimate centromere repeat abundance. Our preliminary analyses of sequence from DNA-binding domains suggest differential rates of evolution in *Zea* homeologs of CenPC and more rapid evolution in CenPC than CenH3 across the Poaceae. We combine information regarding the rate of evolution at these loci with patterns of centromere repeat abundance across the phylogeny in order to gauge the weight of evidence in favor of centromere drive.

Funding acknowledgement: National Science Foundation (NSF)

P8

Common features of chromosome revealed by systematic analysis across species

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Chromosome has significant bearing for recombination, genetic inheritance, and evolution. The rapid accumulation of genomic sequences in different species offers a unique opportunity to systematically characterize chromosomes at the nucleotide base level. Although the size and base composition of chromosomes vary among and within species, systematical analysis across species revealed common patterns of these two basic chromosome features. We discovered that there is an upper limit of chromosome size variation in diploid Eukaryotes with linear chromosomes and variation in chromosome size for eukaryotic genomes (including maize and human) can be viably captured by a single model. The second striking discovery is the degree of freedom of base composition is 1, rather than 3, for 4 nucleotide bases. Further analysis focusing on maize and human indicated that size and base composition of chromosomes are two major factors defining chromosome territories in living cells.

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P9

Comparative analysis of the response to drought stress and recovery irrigation in sorghum and maize

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Drought is a cause of major crop losses worldwide. Sorghum is one of the crops that are better adapted to drought conditions. In comparison, maize is broadly less tolerant of drought, but certain maize varieties, for example the Mexican criollo Michoacan 21, withstand drought better than others. Sorghum and Michoacan 21 exhibit a similar latency mechanism to tolerate drought, slowing vegetative growth before flowering in dry conditions, and rapidly resuming normal growth when drought ends. We want to understand more about gene regulation underlying the response to stress by water deficit in sorghum and the maize variety Michoacán 21, and to find out if they use similar or different molecular mechanisms. We grew plants from each variety under greenhouse conditions. Half the plants were subjected to drought until reaching -2 MPa of soil water potential. The rest were subjected to the same drought stress but were then watered and allowed to recover for 24 hours. In all cases leaf and root samples were taken and RNA was extracted from all samples. We are currently in the process of preparing libraries to be sequenced with Illumina technology. The reads will be mapped to the reference genome of maize and sorghum, in order to quantify transcript expression. To help drive the biological interpretation of the underlying gene expression changes, we have started to define coherent gene modules related to drought stress. We will then use a gene set enrichment method to discover if any of these gene sets respond to drought in our experiments. This will allow exploring a functional response in our experiments without imposing a predefined threshold on the levels of gene expression change. It will be interesting to observe which genes sets respond to drought in a similar or different way in sorghum and maize varieties.

Funding acknowledgement: Consejo Nacional de Ciencia y Tecnologia (Conacyt)

P10

Using Ontologies to Describe Phenotypes in Maize and Across Species

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The phenotypes linked to classic gene models traditionally have been described quite casually, using free text. These descriptions may be informative to human readers but are inefficient for comparing across many genotypes and phenotypes, and they may be completely unusable for computerized comparisons. Across species, traditional phenotype descriptions are even less comparable, due to different naming traditions. For example, the phenotypes “adherent leaf” in *Zea mays* and “fiddlehead” in *Arabidopsis thaliana* are very similar, but this would be impossible to discern based on their names. Consistent, semantically meaningful descriptions of phenotypes are essential to the effective use of high-throughput phenotyping data and bio-informatic approaches to genotype-phenotype analysis. Ontologies provide the structured, semantically enriched vocabulary needed to describe phenotypes for contemporary biology.

This presentation will briefly introduce ontologies and cover how ontologies can be used to describe phenotypes, including a discussion of the differences and similarities (from an ontological perspective) among phenotypes, traits, characters, and character states. It will include a brief overview of how different groups are using ontologies to describe phenotypes (for both plants and animals), with a more detailed description of MaizeGDB’s efforts to create and store ontology-based phenotype descriptions. The presentation will conclude with a discussion of how phenotypes that have been scored using ontologies can be compared across model species to search for similar genotype to phenotypes mappings.

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

P11

The Phenotype RCN Plant Working Group: Ontological Descriptions of Phenotypes Allows Cross Species Comparisons

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The phenotypes linked to classic genes traditionally have been described quite casually, using free text. These descriptions may be informative to human readers but are inefficient for comparing across many genotypes and phenotypes, and they may be completely unusable for computerized comparisons. Across species, traditional phenotype descriptions are even less comparable, due to different naming traditions. For example, the phenotypes “adherent leaf” in Zea mays and “fiddlehead” in Arabidopsis thaliana are very similar, but this would be impossible to discern based on their names. This inability to become aware of equivalent phenotypes misses opportunities for simple discovery that enables the generation of testable hypotheses that relate genotype to phenotype. Consistent, semantically meaningful descriptions of phenotypes are essential to the effective use of high-throughput phenotyping data and bioinformatic approaches to genotype-phenotype analysis. Ontologies provide the structured, semantically enriched vocabulary needed to describe phenotypes for contemporary biology. Here we show how ontologies can be used to describe phenotypes, including a discussion of the differences and similarities (from an ontological perspective) among phenotypes, traits, characters, and character states. We include a brief overview of how different groups are using ontologies to describe phenotypes (for both plants and animals), with a more detailed description of MaizeGDB’s efforts to create and store ontology-based phenotype descriptions. We also show how phenotypes scored using ontologies can be compared across model species to search for genotypes based on phenotypes mappings. Such comparisons will be useful to identify candidate genes associated with known phenotypes in non-model species. This effort is part of a pilot project that aims to create and analyze ontology-based phenotype descriptions across 8 different model and crop plant species.

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

P12

How to Access and Use the New MaizeGDB Website

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MaizeGDB.org is the model organism database for maize. We are rolling out our snazzy new website at this meeting! Have no fear: the data you are accustomed to accessing are still there. In addition, new data and tools have been added. At this poster we will show you how to access standard features and will introduce you to some new features designed to make your data searches easier and more intuitive. Stop by to learn how to access: genes, gene models, mutant phenotypes, genetic and physical maps, contact information for colleagues, the data centers (including a new "Expression Data" center), the new BLAST interface, and much more. We also will demonstrate new features you can use by logging in to the site. During poster sessions we will have computers ready to show you how to find the data you need.

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

P13

MaizeGDB has evolved!

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The focus of genetic, genomic, and breeding research evolves over time, making it necessary to continually redefine the paradigm for data access and data analysis tools. Here we report the reinvention of MaizeGDB, the maize genetics and genomics database, to meet maize researchers' ever changing needs. New, emerging, and prevailing areas of research that guided the reinvention of MaizeGDB include the availability of a well-sequenced reference genome and resequencing information from literally thousands of diverse inbred lines and populations, as well as the emergence of computational tools that enable the execution of, e.g., detailed functional genomics analyses before ever setting foot in the wet lab or research plot. The overall goal of the 2-year MaizeGDB redesign has been to expand 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 and interaction tools) into the MaizeGDB resource. A key component to the redesign has been community involvement. Several community members have volunteered their time and perspectives as beta-testers for the new site and continue to provide valuable insight. In addition the community at large has offered perspectives via email, website feedback, and personal interactions. Here we provide an overview of the new website, updates on new and forthcoming data and data types, and describe the stages of release planned for the new site. To try out the new website now, visit us at http://alpha.maizegdb.org.

Funding acknowledgement: United States Department of Agriculture (USDA)

P14

Maize Reference Genome Sequence Stewardship: Infrastructure to Enable Rapid Access to Genome Updates and Allow Improved Diversity Representation

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The B73 reference genome was assembled from a tiling path of BACs, sequenced to an average depth of 4-6X. As sequencing technologies mature and other genomics techniques are developed and applied to the maize genome, we and the community, as stewards of the maize reference genome, will need to manage updates to the reference assembly. The Genome Reference Consortium (GRC), the group responsible for the upkeep of the human, mouse and zebrafish reference assemblies, has developed and deployed a data model and established standardized operating procedures to manage assembly updates[1]. The data model enables alternate genomic assembly representations to accommodate genomic diversity. In this model, alternate versions of genome regions too complex to be adequately represented by a single chromosome path are included as accessioned scaffold sequences along with their corresponding alignments to the chromosome. In addition, the model introduced the concepts of assembly patches and minor releases that enable researchers to create and access genome updates between full assembly updates, without disruption to chromosome coordinates. The GRC also has tools that allow the user community to directly report assembly issues and provide feedback into the process of assembly curation. Although only vertebrate genomes are currently managed by the GRC, the model is applicable to any genome with a tiling path of accessioned component sequences. We are currently working with NCBI to populate an instance of the GRC data model with the B73 reference genome to determine how best to make use of this robust system. Here we report our progress and outline some unique complications posed by the B73 reference genome sequence as well as our efforts to overcome these challenges.

[1]PLoS Biol. 2011 Jul;9(7):e1001091. doi: 10.1371

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), National Institutes of Health (NIH) / National Center for Biotechnology Information (NCBI)

P15

MaizeGDB Genome Browser

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MaizeGDB (http://www.maizegdb.org) is the database for maize genetics and genomics. The latest assembly of the reference genome, B73 RefGen_v2, has been available on the MaizeGDB Genome Browser since May 2010 and has been thoroughly integrated with structural and functional annotations. Displayed using GMOD's GBrowse2, the users of the MaizeGDB Genome Browser now how more control over how they view annotations including scrolling, zoom, and centering, subtrack options for grouping related data sets, as well as the ability to upload third party annotations. New data sets include Panzea HapMapV2 and KNOTTED 1 Binding Regions from the Hake Lab. Some new and upcoming tracks include an RNA-SEQ gene model expression track and B73 RefGen_v3 when the data set is released by GenBank. New features have been developed at MaizeGDB including a feature that converts the length being viewed on the Genome Browser from base pairs to Centimorgans. Also now available is a new annotation display that shows expression data based on genomic annotations. Check out our expression glyph at http://gbrowse.maizegdb.org/gb2/gbrowse/maize_v2/?label=kaeppler

Funding acknowledgement: United States Department of Agriculture (USDA), Iowa State University

P16

Compare Identity By Sequence Relationships of the Ames Diversity Panel using TYPSimSelector

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Maize genetic diversity has been exploited by mankind for 10,000 years. Scientific approaches applied to it by breeders for over a century transformed it into the world's number one crop. Maize genomic diversity provides a rich resource of interest to evolutionary and population geneticists, constitutes the materials available for crop improvement, and enables investigation of the relationships between genetic variation and gene function. The Ames diversity panel is a set of approximately 2,500 inbred maize accessions primarily made available via the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. The panel was interrogated with over 680,000 SNP markers to establish identity by sequence (IBS) relationships (Romay et al., 2013). These relationships are being compared using SQL queries in a MS SQL database developed by M. Millard at the NCRPIS. It has proven useful for examining relationships between lines based on user-defined IBS criteria, including identifying those most similar or divergent from a given line, and is currently being used to aid in management decisions for *ex situ* conservation of germplasm. A tool called TYPSimSelector is under development at MaizeGDB to enable web access by the maize research community. We invite you to visit this poster as well as the Diversity Data Center at http://alpha.maizegdb.org to learn more and try it out!

Funding acknowledgement: United States Department of Agriculture (USDA), Iowa State University

P17

Gene Expression Analysis Tools at MaizeGDB

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The completion of the maize genome sequence in 2009 has created both significant challenges and opportunities for maize researchers. The advent of next generation sequencing only added to this deluge of data. It is truly a great time to be a biologist! However, the opportunities for understanding cellular processes underlying maize's phenomenal productivity can only be realized if functional genomics software tools (FGSTs) are available to reduce the complexity of multimillion point data sets into manageable images and/or concepts that are both intuitive and which allow in-depth analysis. Currently, MaizeGDB is hosting numerous large gene expression data sets, and more will be deposited in the near future. Fortunately for maize researchers, free public domain FGSTs have been developed for other biological systems and their implementation at MaizeGDB or through collaborations with MaizeGDB can be accomplished with a moderate amount of effort. In this poster, we describe efforts at MaizeGDB to make these tools available to users at all levels, and our ongoing effort to redesign our gene expression data center page as part of our MaizeGDB user interface redesign. Additionally, new data sets (tools??) that enhance the reach of these tools will be described. Please stop by our poster and let us know what expression tools you would like to see at MaizeGDB. Input from our users is our best source of feedback for what expression analysis tools MaizeGDB should be developing.

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P18

Pathways at MaizeGDB - Strategies for curation and data sharing.

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Functional genomics became a reality for maize with the release of the draft sequence of 2.3 GB B73 inbred line in 2009. Gene expression datasets both at MaizeGDB and in other databases contain largely predicted functions. These rely on evidence compiled by UniProt and the GO Consortium, and where TAIR is the main contributor of higher plant data to these resources. We report here a new pathways curation effort at MaizeGDB and that leverages two distinct BioCyc Pathway-tools representations. One, CornCyc, is a very high stringency product generated by the Plant Metabolic Network project, which relies on the highly curated AraCyc. The other, MaizeCyc, generated by the Gramene project, was less stringently computed and with greater likelihood for assigning a putative gene function to a gene model of interest. In addition to curation tools included in the Pathway Tools software suite, we have developed a Gene Ontology (GO) annotation module that supports community curation and provides robust validation via the NCBO portal using RESTful webservices. Scripts have been developed that import GO annotations from MaizeGDB to the object-oriented BioCyc databases.

We will initially curate pathways selected for relevance to important agronomic traits, availability of data in maize, especially mutants, and any suggestions from the MaizeGDB Working Group. We are exploring several textmining approaches to enhance our potential for curation.

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P19

Metabolic Pathway Resources at MaizeGDB

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Two maize metabolic networks are available at MaizeGDB: MaizeCyc (http://maizecyc.maizegdb.org, also at Gramene) and CornCyc (http://corncyc.maizegdb.org, also at the Plant Metabolic Network). MaizeCyc was developed by Gramene, and CornCyc by the Plant Metabolic Network, both in collaboration with MaizeGDB. MaizeCyc and CornCyc are both based on B73 RefGen_v2 filtered gene set models, and offer visualization and analysis capabilities of Pathway Tools developed by SRI. Their pipelines for enzymatic function assignment indicate some differences: MaizeCyc is mainly based on the exonerate scores generated via the Ensembl XRef pipeline, whereas CornCyc employs a scoring matrix based on performances obtained using BLAST, CatFam, and Priam. As a result of these different assignment criteria, the metabolic networks differ in coverage and confidence levels. Here we present some statistics from both metabolic networks, provide snapshots of various views and analysis tools available in Pathway Tools, and show examples of how these tools and resources can be used to derive biologically-meaningful hypotheses.

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P20

CycTools: An Interface for Exploring and Updating BioCyc Databases

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The BioCyc collection contains over 2,038 Pathway/Genome Databases (PGDBs), including the MaizeCyc (@MaizeGDB and @Gramene) and CornCyc (@MaizeGDB and @ the Plant Metabolic Network) metabolic networks. Pathway Tools is a navigation and analysis platform for PGDBs. While Pathway Tools provides an interface that allows a user to curate a PGDB, lack of remote or concurrent write access and a limited ability to batch load data into the PGDB can present a challenge when multiple curators attempt to update a single database instance. We present a new tool to allow facilitated curation access, including the ability to perform remote batch uploads to a PGDB. CycTools is a Java-based interface for interacting with the BioCyc family of databases. By providing both read and write access to a PGDB, this tool can be used as an alternative to the Pathway Tools interface for updating a PGDB. By allowing batch uploads, curators are freed from manual, error prone entering of data into the pathway tools interface. Data can be collected and validated using external tools and later applied to a database as a single discrete operation. This ability enables curators to work with a database without requiring them to learn the underlying database structure. It also allows the same operation to be performed to multiple PGDBs. We show how the batch upload function can be used to update two PGDBs, MaizeCyc and CornCyc, representing the same organism using literature-based GO annotations provided by the MaizeGDB Team.

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P21

Functional annotation of B73 gene models: A machine learning approach

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

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

P22

A SNP-based high-throughput genetic mapping data analysis tool for mapping mutants and QTL

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Genetic mapping provides basic information about the location of markers or genes along the chromosome. A well-constructed genetic map is prerequisite to determining the locus responsible for mutants in any organism. In maize, genetic maps and markers are important for additional studies such as marker assisted breeding or determining the chromosomal location of QTL responsible for a certain trait. A large number of SNP markers have been identified by genome sequencing, and a project undertaken at Iowa State University (ISU) has created a set of ISU-SNP markers. These 1016 markers are derived from inbred lines B73 and Mo17 but useful across a range of inbred lines. They are well suited for initial, high-throughput rough mapping of mutants or QTL of interest using a Bulked Segregant Analysis (BSA) approach. The Genomics Technology Facility (GTF) at ISU analyzes the ISU-SNP markers in a high-throughput manner using Sequenom-based SNP typing assays. Data produced by this analysis contains a wealth of information that can help scientists identify a chromosomal region for fine mapping studies. To help researchers obtain as much information as possible from ISU-SNP BSA data, we have created a web tool that enables scientists to analyze and manually inspect the results of this high-throughput analysis. The tool processes the data and provides different outputs such as raw data, processed data including highlighting functions for putatively linked markers, a set of graphs for easy visualization of linkage, and a summary table of significant markers and regions. The tool is freely accessible at http://gokul.gdcb.iastate.edu/ev_lab/bsa_analysis.php.

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

P23

Comparative transcriptomics as a tool for the identification of root branching genes in maize

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The root system is fundamental for plant development and is crucial for overall plant growth. A major determinant of root system architecture is the initiation of lateral roots. Although increasing knowledge is achieved in the dicotyledonous plant *Arabidopsis thaliana*, very little is known about the genetic and molecular mechanisms involved in lateral root initiation in major crop species, generally monocotyledonous plants. The existence of both similarities and differences at the morphological and anatomical level between different plants raises the question whether the regulation of lateral root initiation is conserved through evolution. Here, we developed a method to synchronize the induction of lateral roots in primary and adventitious roots of *Zea mays*, and used it to perform a genome-wide transcriptome analysis during lateral root initiation. We found a high level of correlation in gene regulation between primary and adventitious lateral root formation. Further, a comparison with data from Arabidopsis revealed a core of genes involved in lateral root initiation that seems to be conserved across the Angiosperms. We conclude that conserved regulatory mechanisms exist for lateral root initiation in maize and Arabidopsis, a finding that might encourage approaches attempting to extrapolate knowledge obtained in the model plant Arabidopsis to crop species at the level of root branching and root system architecture.

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P24

Comprehensive Analysis and Evolutionary Conservation of Alternative Splicing Events of Plant SR Proteins

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The high frequency of alternative splicing among plant Serine/Arginine-rich (SR) family of proteins have been linked to important roles in gene regulation during development and in response to environmental stress. In this report, we have searched and performed manual annotation of all the SR proteins in the genomes of maize and sorghum. The experimental validation of gene structure by RT-PCR analysis revealed, with few exceptions, that SR genes produced multiple isoforms of transcripts by alternative splicing. Despite sharing high structural similarity and conserved positions of the introns, alternative splicing profiles of vast majority of SR genes are not conserved between maize and sorghum. These include many transcript isoforms discovered by RT-PCR and not represented in extant EST collection. We demonstrate the occurrence of several isoforms of maize and sorghum SR mRNAs and that these isoforms display evolutionary conservation of splicing events with their homologous SR genes in Arabidopsis and moss. Most importantly, our data indicates an important role of both 5' and 3' untranslated region (UTR) in the regulation of SR gene expression. These observations potentially show importance of this process in evolution and adaptation of plants to land.

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P25

Computational identification of conserved root hair elements in maize

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There are numerous well-studied promoters known to express in specific cells or domains but motifs that drive expression specific to one individual cell type are rare. The root hair element (RHE) is one such motif that was originally discovered in *Arabidopsis thaliana* but has been found to be conserved among various plant lineages, including maize. Previous studies have shown that cloned RHEs from several different species can drive GFP expression that is specific to root hairs in *Arabidopsis*. Though the RHE has been found to give rise to cell-specific expression, there are over 700 instances of its sequence (WHHDTGNNN(N)KCACGWH) within the *Arabidopsis* genome and ~8700 in the maize genome. Our work seeks to distinguish functional motifs from false ones via computational identification of conserved genes or gene pairs that have either maintained in the expected syntenic position or lost their associated RHEs. Through this, it has been possible to cut through the ambiguity of the motif itself and locate those few RHEs likely to have functioned in the plant ancestor and still function today. In other instances, loss of the regulatory sequence from one homeolog of a maize pair has led to a corresponding loss of expression specificity. An orthologous gene that is root hair specific in *Arabidopsis* may be expressed elsewhere in maize if it has lost its RHE. The conservation of regulatory elements over time can elucidate how a cis-acting sequence has been co-opted multiple times throughout evolutionary history to drive specific expression of important genes. Using the revolutionary software MotifView, a beta-test application for the CoGe suite of comparative genomics tools, we have located associated RHEs in maize genes orthologous to known root hair genes in *Arabidopsis.* We hope for better root-epidermis and root hair expression data in maize so our *Arabidopsis*-maize comparison can continue.

Funding acknowledgement: National Institutes of Health (NIH)

P26

Detecting Causal Genes for Maize Agronomic Traits Using Co-Expression Networks

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Many agronomic traits in maize are known to be complex phenotypes with genetic contributions from multiple loci. Deciphering this relationship between genotype and phenotype has long been hindered by a complex genome and an astounding amount of genetic variation. While classic gene mapping approaches and advanced mapping populations have been successful in isolating genes with large phenotypic effects, they many times fall short in identifying genes within loci of smaller effect due to limited resolution. Even with strong signals of statistical association, candidate regions contain possibly hundreds of genes leaving the majority of causal genes unidentified without additional genetic analysis. The advance of high-throughput sequencing technologies for measuring gene expression has now made it possible to create gene networks from transcriptional data, and use them to examine putative function and co-regulatory relationships on a genome wide scale. We are developing computational methods for integrating candidate loci generated from mapping populations with functional information derived from maize co-expression networks. This approach leverages network structure to identify causal genes within regions of the genome linked to elemental accumulation by incorporating functional information learned from co-expression networks. We illustrate how co-expression networks contain biologically relevant information and when coupled with joint linkage-association data, can be used to detect causal variants at a higher resolution than conventional approaches.

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P27

Does combining different detection algorithms improve the robustness of whole-genome prediction when a mixed large and small underlying genetic architecture is present?

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Current genome-wide evaluation methods work best on specific genetic architectures. For example, the nonlinear Bayesian variable selection method (BayesB) works well when there are many small-effect alleles, and genomic linear method (GBLUP) works well when there are a few large-effect alleles in the population. In theory, heterogeneity of performance with different data types can be addressed by combining algorithms. We are assessing genotype/phenotype association prediction accuracy when evaluating a mixed large and small underlying genetic architecture. Varying combinations of detection algorithms will be evaluated for their accuracy in replicating genotype/phonotype associations in several types of known-truth simulated datasets.

Ultimately the results of this study will be utilized in identifying the most efficacious algorithm features for the analysis of genotype/phenotype associations in whole-genomes with mixed large and small underlying architecture as well as facilitating the development of optimized algorithms for the same purpose.

Funding acknowledgement: United States Department of Agriculture (USDA)

P28

DsgMapper: A pipeline tool for the identification of Ds-targeted sequences from next-generation sequencing data

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Next-generation sequencing (NGS) technology is an unprecedented high throughput and cost-effective way of sequencing genomes. In our NSF-PGRP-funded project, we developed a pipeline tool for the analysis of NGS data to identify *Ds*-targeted sequences and, thus, generate a sequence-indexed *Ds* library. Our transgenic *Dsg* element is marked with the jellyfish green fluorescence proteins (GFP), allowing amplification of *Dsg*-adjacent sequences with nested PCR primers based on GFP and a 5-bp region at the end of *Ds* (also known as the *Ds* identifier). DNA samples are sheared and amplified in multiple phases so as to enrich for *Dsg*'sand their adjacent insertion sites. A 3-D pooling strategy is adopted to further increase throughput. Each sample is placed in a 3-D well, where every dimension is barcoded before sequencing starts. Our raw data consist of reads assigned to different libraries according to their barcodes. The pipeline aims to: map reads back to their original wells (i.e., deconvolute the 3-D pool); and localize *Dsg* insertion sites in the maize genome. First, in each library, the pipeline filters out reads unrelated to *Ds*-adjacent sequences by checking accordance between reads and the sequence of the PCR primer plus the *Ds* identifier. Retained reads are imported into MySQL database, with each database table linked to one library. Reads are merged according to well coordinates from related 3-D libraries and ranked by their copy number after grouping by sequence. Ideally, the top ranked sequence in a specific well should correspond to the real *Dsg*. However, due to the presence of endogenous *Ds* elements and possible sequencing errors, top-ranked candidates have to be carefully inspected to identify the real *Dsg*. An alternative to 3-D merging is to rank reads in individual libraries and pick reads with top ranks in all three dimensions, which complement the merging method yet requires empirical thresholds. Last, *Ds* insertion site junction sequences are mapped to the maize genome using a local version of BLAST. This pipeline tool is suitable for massive NGS data manipulation in similar scenarios.

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P29

Estimating allele-specific expression levels from RNA-Seq data

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RNA-Seq analyses have the potential to estimate the transcript abundances of different alleles of the same gene. However, estimates of allelic abundances may be biased when reads are mapped to a reference genome. Reads of transcripts with high similarity to the reference allele map more efficiently than reads with low similarity to the reference allele. We describe the magnitude and prevalence of this bias in an analysis of RNA-Seq data from reciprocal B73xMo17 hybrids mapped to the B73 reference genome. To reduce mapping bias within the hybrids, we describe a multi-step data processing strategy that utilizes RNA-Seq data from the inbred parents. The data processing strategy improves estimates of allele-specific expression levels, thereby strengthening downstream analyses.

P30

Estimating the proportion of variation explained by rare variants for six complex traits in whole genome sequence-based studies

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As the frontier of human genetic studies have shifted from genome-wide association studies (GWAS) towards whole exome and whole genome sequencing studies, we have witnessed an explosion of new DNA variants, especially rare variants. An important but not yet answered question is the contribution of rare variants to the heritabilities of complex traits, which determine, in part, the gain in power from rare variants to discover new disease-associated genes. Here we present theoretical and empirical results on this question.

Our theoretical study was based upon the distribution of allele frequencies incorporating mutation, random genetic drift, and the possibility of purifying selection against susceptibility mutations. It shows that in most cases rare variants only contribute a small proportion to the overall genetic variance of a trait, but under certain conditions they may explain as much as 50% of additive genetic variance when both susceptible alleles are under purifying selection and the rate of mutations compensating the susceptible alleles (i.e. repair rate) is high.

In our empirical study, we estimated the proportion of additive genetic variances of rare variants contributed to the total phenotypic variances of six complex traits (BMI, height, LDL-C, HDL-C, triglyceride and total cholesterol) using whole genome sequences (8x coverage) of 962 European Americans from the Charge-S study. The results show that the estimated variance of rare variants (MAF<=1%) ranged from 2% to 8% across the six traits. However, the standard errors (s.e.) of the estimated variance components from rare variants are relatively large compared to those of common variants. Using HDL-C as an example, the estimated variance are 0.08 (s.e. 0.10), 0.05 (s.e. 0.05) and 0.58 (s.e. 0.05) for rare, low-frequency and common variants, respectively.

P31

Exploring maize diversity with Gramene

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Gramene (www.gramene.org) is a curated resource for comparative functional genomics in crops and model plant species. Its strength derives from the application of a phylogenetic framework for genome comparison, and by the integration of genome annotation and functional data using ontologies. In addition to *Zea mays*, the current release (Build 36) includes 22 complete reference genomes, with strong representation of both monocots and dicots. For maize this release features the new HapMap2 data (Chia et al. 2012. Nature Genetics 44, 803–807), which incorporates 55 million SNPs and indels identified in a collection of 103 pre-domesticated and domesticated varieties, including a representative from the sister genus, *Tripsacum dactyloides* (Eastern gamagrass). Gramene offers a variety of ways to explore these data. Population views summarize allele frequencies as well as individual genotypes for each line. Gramene also provides results of the Ensembl Variant Effect Prediction (VEP) method, which uses Sequence Ontology terms to classify genotypes with respect to functional impacts on transcript structure (e.g. stop_gained). These classifications are viewed directly in the genome browser, as well as displayed in the context of protein structural domains. To enable powerful custom queries and downloads, the HapMap2 and VEP data are integrated into BioMart. Use-cases for BioMart searching will be demonstrated. The current release also features an update of the MaizeCyc pathways database to version 2.0.2. In collaboration with the Reactome Project, we have released the beta version of Plant Reactome, a new platform for the comparative analysis of plant metabolic and regulatory networks, currently prototyped in rice. Gramene is a product of close collaboration with the Ensembl Plants project (plants.ensembl.org) and the Ontario Institute for Cancer Research (OICR). Gramene is funded by NSF grant IOS- 1127112

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P32

Functional analysis of the maize phyllosphere microbiome

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A primary habitat for microorganisms is the phyllosphere, the leaf surface of plants. Metagenomics techniques allow these microorganisms to be taxonomically classified and functionally analyzed in order to identify these microbes and to determine their function and how this function equips these microbes for their unique environment. Utilizing iPlant Collaborative, 32 metagenomic samples (of 4,000-44,000 reads) from the phyllosphere of NAM founders were analyzed in order to determine taxonomy and to provide functional analyses. Taxonomy was identified using BLAST, and further taxonomic classification and functional analysis was determined using MEGAN4 via the SEED and KEGG classification mechanisms. These methods highlight the functional similarities between the samples in relation to the different microbial composition of the samples as well as allow relationships to be inferred regarding the interactions between the microorganisms and the plant. For a subgroup of these phyllosphere metagenomic samples, in addition to a marine sample, we calculated the Canberra Stability Indicator, a metric that quantifies the disorder among pairs of ranked lists and is a useful technique in comparing ranked lists in functional genomics. Individual enzymes within each sample were numerically ranked according to their abundance. The stability indicator was calculated for differing numbers of enzymes (top 100, 250, 500, and 1000 hits) between the samples, quantifying the stability of the enzymatic composition of each of the samples, i.e. the functional stability of the samples. Values ranged from 0.36 to 1.30, and as the size of the ranked lists increased, the stability between the samples also increased. These bioinformatics methods can potentially lay the foundation for future applied breeding efforts that target the growth of microorganisms with specific functional capabilities.

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P33

Gene Family Loss and Gain During the Evolution of Flowering Plants

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Angiosperms are quite variable in their nuclear genome sizes, chromosome numbers and rates of genomic rearrangement, but changes in genic content are relatively few. Thus, studying the genetic basis for the great variation in developmental, morphological or physiological features among different species is an important topic in comparative genomics. However, precise genome comparison among plants is challenging because of the their genomic complexity and lability. In this study, we investigated gene family content differences in four grass genomes and four Rosid genomes through a two-step MCL process. This approach first constructs gene families within each species, then exemplars from each gene family are chosen and clustered into cross-species groups. Our study demonstrated that, among the 16,691 gene families that contain more than one member, 4678 families (28%) are shared by all of the investigated Angiosperms. The dating of gene family loss and gain events showed that the rates of gene family content change are not constant across different plant lineages. We also found several functional categories that are enriched in the dynamically changing gene family sets. For instance, gene families that were lost by grasses after their split with Rosids are enriched for those involved in development and stimulus response. In contrast, the most common new gene families were the same for Rosids and grasses, although acquired independently, and were primarily those involved in various regulation processes. This study provides insights into the pattern of gene family evolution during the history of flowering plants, and suggests a functional and selective explanation for this phenomenon.

P34

Gene Regulatory Change in Maize Domestication

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Gene expression differences due to changes in both *cis*- and *trans*-regulatory elements have been shown to influence a number of domestication phenotypes in plants, including maize. We used mRNA sequencing to study gene expression differences in six maize and nine teosinte inbred lines and 29 of their maize-teosinte F1 hybrids. Total RNA was extracted and barcoded strand specific RNA sequencing libraries were produced for three tissues: immature ear, seedling leaf, and seedling stem (including the shoot apical meristem). A pipeline was then developed to assess expression of maize and teosinte alleles using the reference B73 genome, whole genome sequencing reads from the inbred lines, and RNAseq reads from inbred lines and F1 hybrids. We determined *cis*- and *trans*-regulatory change with F1 hybrid and parent inbred line allele specific expression ratios, which were calculated using RNAseq read depth at segregating SNPs attributable to the maize and teosinte parents. Expression ratios from F1 hybrids, where the maize and teosinte allele are expressed in the same *trans*-acting environment, were used to directly infer *cis*-regulatory change. The difference between parent inbred line allele specific expression ratios, representing total regulatory difference, and F1 hybrid ratios were used to infer *trans*-regulatory change. An overall maize-teosinte analysis of regulatory change was done by summing maize and teosinte read depths at segregating sites for all crosses on a gene by gene basis. Genes in the overall analysis showed frequent regulatory differences, with a large number of genes also showing heterogeneity among the 29 maize-teosinte expression ratios. Heterogeneity among the expression ratios was expected due to the multiple maize and teosinte alleles used in the experiment. Genes with consistent expression bias for either the maize or teosinte allele across multiple assays are candidates for involvement in domestication.

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P35

Genome-wide comparative analysis within Sorghum sect. Eusorghum using a next-generation sequencing approach

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Recently, there have been initiatives for the development of perennial grasses for use as alternative crops on both current agricultural lands, in addition to less arable lands that do not currently support crop production. Sorghum, a robust and morphologically diverse genus of herbaceous grasses, contains both perennials and annuals, and therefore, provides an excellent model system for the study of perenniality. The cultivated annual grain S. bicolor is well characterized, and a high-quality genome sequence is available (BTx623). Additionally, S. bicolor forms fertile hybrids with its wild perennial relative S. propinquum, making these two species a good model for studies aimed at elucidating the genetic underpinnings of traits associated with perenniality. To this end, we have generated over 77 Tb of quality trimmed/filtered (90% $\geq$ Q30) genomic sequence for S. propinquum USDA accession PI653737, S. propinquum (accession courtesy of Bill Rooney) and five S. bicolor lines (Shanqui Red, Tx7000, SC56, RTx430, B35) using Illumina HiSeq 2000 technology and assembled these data to the S. bicolor BTx623 reference (13.52x average coverage). These data allow for both an interspecific and intraspecific dissection of the genetic differences within Sorghum annuals and perennials. The genomic distribution of SNPs reveal that over 68% are within upstream/downstream promoter regions, and only 1.5% in coding regions of the genome. These results suggest that the vast majority of SNPs likely contribute to expression differences that lead to morphological change within the genus Sorghum. In addition to genome distribution of SNPs and indels, presence/absence variation of protein coding genes will be shown. These data allow for future analysis of candidate loci and variants associated with domestication traits within the genus.

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P36

Genome-wide genetic variation between the French FV2 inbred line and the B73 reference inbred line

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Maize genetic variation has long been analyzed using molecular markers covering the whole genome and by comparing the sequence of selected orthologous regions. These studies revealed an exceptional structural diversity, including copy number variation (CNV) and presence/absence variation (PAV). The extent of this structural variation has recently been extended at the whole genome scale using high throughput technologies such as microarray hybridization or 'next generation' sequencing. However, maize structural variation has been characterized mainly for American lines and a few Chinese lines, and very little is known about its extent in the European germplasm.

To extend the diversity addressed for structural variation, we resequenced the French maize FV2 inbred line, which originates from a traditional open pollinated European flint variety and played a key role in European breeding programs over the past 50 years. We generated paired-end Illumina sequences to reach a 70x sequencing depth of the FV2 genome. Mapping of these sequences onto the B73 reference genome sequence uncovered 8,000,000 SNPs. Using a reference-guided assembly approach, we also indentified 42,000 regions that are present in the FV2 genome and absent from the reference B73 genome sequence. These regions cover 16 Mb of genomic sequence and are mainly low copy. We discuss these results and compare them to the structural variation previously described for other maize inbred lines.

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P37

Genomic data processing for ancient maize data

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The advent of high throughput sequencing has revolutionized the ancient DNA field. A significant amount of data can be obtained from archeological samples containing as little as 1% of endogenous DNA with a modest sequencing effort. However, the resulting reads must undergo tight filtering and quality control. Ancient DNA has undergone biochemical modifications (namely C->T deamination) and extensive fragmentation (resulting in fragments that are smaller than the read length and have varying lengths). I will present a pipeline that has been optimized for filtering ancient maize data.

P38

Grass Gene Regulatory Information Server, GRASSIUS

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I will be presenting a poster on the grass transcription factor database GRASSIUS, and its evolving utility as a repository for the information about maize gene regulatory networks that is being produced by the maize transcription factor ORFeome (TFome) project.
The Grass Regulatory Information Server GRASSIUS has served as a database for information pertaining to gene regulation in the grasses. Currently, there is information for transcription factor families in maize, rice, sorghum, sugarcane, and brachypodium. It will also serve as a repository for cis-regulatory information in GrassPROMDB, a database of curated promoters. The GRASSIUS Regulatory Grid Explorer (GRG-X) provides a place to visualize the gene regulatory networks.
The TFome project to clone all maize transcription factors and coregulators into entry vectors is currently underway, and will be followed by subcloning into yeast one-hybrid and yeast two-hybrid vectors for protein-protein and protein-DNA interaction studies. The findings of these experiments will be hosted at the GRG-X and will provide the scientific community with a one-stop shop resource to view transcriptional regulatory interactions that has heretofore been lacking. The entry clones, as well as the one hybrid and two hybrid clones will be made publically available.
This novel information on gene regulatory interactions in maize will greatly increase the utility of GRASSIUS to the scientific community, and the TFome collection, when released to the public through GRASSIUS, will accelerate the scientific community's discovery of new transcriptional regulatory motifs in maize.

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P39

GSV/mGSV: Web-based Genome Synteny Visualization Tools for Customized Data

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Web-based synteny visualization tools are important for sharing data and revealing patterns of complicated genome conservation and rearrangements. Such tools should allow biologists to upload genomic data for their own analysis. This requirement is critical because individual biologists are generating large amounts of genomic sequences that quickly overwhelm any centralized web resources to collect and display all those data. We have developed a web-based synteny viewer package, GSV/mGSV, which was designed to satisfy the above requirement. GSV (http://cas-bioinfo.cas.unt.edu/gsv/) allows pair-wise genomic comparison, while mGSV (http://cas-bioinfo.cas.unt.edu/mgsv/) extends the comparison to multiple pairs of genomes. Users can upload their own genomic data files for visualization. Multiple genomes can be presented in a single integrated view with an enhanced user interface. Users can navigate through all the selected genomes in either pairwise or multiple viewing mode to examine conserved genomic regions as well as the accompanying genome annotations. Besides serving users who manually interact with the web server, Web Services were also provided for machine-to-machine communication to accept data sent by other remote resources. The entire GSV/mGSV package can also be downloaded for easy local installation.

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P40

HelitronScanner: A two-layered local combinational variable approach to generalized Helitron identification

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Helitrons, new rolling-circle eukaryotic transposons discovered in plant and animal species, have been implicated in processes of great evolutionary significance, such as gene duplication, exon shuffling, and horizontal transfer. Identifying *Helitrons* is a challenge because, unlike other DNA transposable elements, *Helitrons* have no terminal repeats and do not create target site duplications. Here we present a two-layered Local Combinational Variable (LCV) approach (HelitronScanner) for generalized *Helitron* identification. LCV represents either patterns of nucleotide sequences or the combinations/associations of such sequence patterns. The first layer extracts location-nonspecific LCVs (n-LCV) in a known *Helitron* set and then creates a distribution matrix of these n-LCVs matching against *Helitrons*. The second layer draws location-specific LCVs (s-LCV) from the distribution matrix. This two-layered procedure is applied to new sequences comparably. In HelitronScanner, n-LCVs represent sequence patterns shared by *Helitrons* and s-LCVs constitute the associations of these patterns within *Helitrons*. Both dry lab and wet lab verifications were conducted to reduce the false positive rate. We first compared *Helitrons* identified by both HelitronScanner and HelitronFinder (one of our previous algorithms for *Helitron* detection). HelitronScanner detected more *Helitrons*, including those also identified by HelitronFinder. *Helitron* insertion polymorphisms were identified by (i) matching flanking sequences against genome sequence data of 6 maize inbred lines and (ii) PCR assays of *Helitron* vacant sites in multiple maize inbred lines. These verification feedbacks allow HelitronScanner to be iteratively improved in order to achieve high accuracy and convenience in genome-wide *Helitron* identification. HelitronScanner has been run successfully on a wide array of plant genomes.

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P41

Historical Changes in Repetitive Sequence and Total Genomic Content In Maize and Related Grasses

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Changes in DNA sequence and structure are frequent in the history of maize, though our understanding of structural changes is much less thorough than sequence differences. We set out to document the structural modifications in the maize genome that have occurred in populations locally adapted to different environments. In particular, we are interested in the fluctuations of repetitive content in populations of maize, its progenitor teosinte, and the closely related grass *Tripsacum dactyloides*. We utilized low coverage Illumina sequencing and the detailed annotation of the maize genome to better understand the evolution of three classes of repetitive sequence: transposable elements (TEs), heterochromatic knobs, and tandem centromeric repeats. After correcting for genome size variation in these populations, we compared abundance of these three repetitive classes in a diverse panel of 500 different maize landrace, teosinte, and *Tripsacum* individuals. Our results show a number of environmental trends, most notable of which was clinal variation in repeat abundance with altitude. Surprisingly, different classes of repeats showed opposing clinal patterns, suggesting that both simple neutral models and models of selection against genome size are insufficient to explain the data.

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P42

Identification and characterization of deeply conserved plant non-coding sequences

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Conserved non-coding sequences (CNSs) are phylogenetically-conserved footprints under purifying selection. In mammals, conserved noncoding elements (CNEs) are both very long and very highly conserved. Amongst placental mammals ~14,000 CNEs exist that are at least 100 bp long and share 100% sequence identity (Stephen et al., 2008). A subset of CNEs are detectable between mammals and fish (400 Myr divergence). In contrast, in plants CNSs are much shorter and only recently have been detected between species as distantly related as *Arabidopsis* and grape (117 Myr divergence) (Baxter et al., 2012).

Starting from a set of 16978 CNSs conserved in Poaceae, we used the Musa genome to identify 116 CNSs conserved in the commelinid monocotyledon lineage (d'Hont et al., 2012). In parallel, we identified 211 CNSs conserved between *Arabidopsis* and Columbine. Fifty of these CNSs were found to be conserved in *Amborella trichopoda* (~170 Myr divergence), and thus represent pan-angiosperm CNSs.

Deeply conserved pan-commelinid and pan-eudicot CNSs are highly associated with genes involved in transcription regulation and development, with half of deep eudicot genes being annotated with the GO term "transcription factor activity". Many of these CNS sequences likely correspond to transcription factor binding sites, and both WRKY-binding sites and the motif "CATGTGA" were found to be statistically enriched in both the pan-commelinid and pan-eudicot CNS collections. Experimental evidence for a cis-regulatory role for ten of these CNSs has been published, and another CNS (present in the 3' UTR of THIC), has been shown to be a riboswitch (Wachter et al., 2007). Other CNSs are likely to play a role in regulating transcript levels of RNA-binding proteins via alternative splicing.

After both the pre-grass and maize whole genome duplication events, genes associated with deep-commelinid CNSs were found to be more likely retained as duplicates than genes with less deeply conserved CNSs.

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P43

Image Processing and Segmentation of Lesion Mimic Mutants

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The lesion phenotypes expressed on maize leaves are complex. The lesions from the different mutations differ in their size, structure, growth rate, distribution, and many other dimensions. To better understand the network of genes, processes, and reactions that produces these very complex phenotypes, we need to be able to quantitatively characterize these differences.

We photograph leaves with lesions from different mutants, such as *Les2*, *Les4*, and *Les7*, repeatedly back-crossed onto Mo20W, W23, and M14. These images are collected under standard conditions that simplify their processing. We mask the leaf from the background information and then segment each lesion from the leaf. Our goal is to collect dimensional information for each lesion from each image for statistical analysis.

Segmentation is the process of algorithmically dividing an image into its component parts. We use MatLab and its image processing toolbox functions, including *bwboundaries* and *bwperim*. These functions use a black and white transformation of the original masked color image, and search for adjacent black pixels that delimit connected regions on the image. The quality of the segmentation depends in part on how we threshold the transformation from the color to the black and white image. Some lesions are nested inside each other; *bwboundaries* can identify these as well. Once the lesions are segmented, we mark each segmented lesion with a pink boundary on the original color image to rapidly check how well we have tuned the various parameters. We are also testing segmentation by other methods, such as by color; and combinations of color and edge detection. The matrix of segmented lesions is passed to other MatLab functions for dimensional analysis. We plan to characterize the phenotypes by analyzing populations of segmented lesions.

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P44

Laboratory Information Management for Computational Experiments

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We are characterizing the phenotypes of the disease lesion mimic mutants. To do this, we have taken photographs of leaves and are creating solid computational ways to distinguish one type of lesion from another by processing the photographs into lesions. Since there are thousands of photos and many different types of lesions, we need a concrete way to determine the best image processing methods and parameters to use; and to efficiently store both this information and the resulting quantitative data. We have therefore designed and are implementing a pipeline system that processes the images and records how the processing was done. For each computation, the pipeline will fetch code, parameter, and function files from a version control system; images from disk, or data from a database; will record how the computation was done in a database; and will store the resulting output either on disk or in the database. The system will track each computational experiment and its components, allowing us to replicate any computation and analyze the results from many. The pipeline itself is being written in Perl, and will organize MatLab computations from function libraries and parameter files. Code, parameter, and script files will be stored in Bazaar, a distributed version control system. Records of the computations will be stored in a MySQL database. We plan to use the system to track biochemical experiments as well. In this way, we are transforming the qualitative data of biologists into quantitative data that can be stored in a database and quickly referenced for research.

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P45

Large-scale identification of sequence-indexed *Mu* insertion sites in MTM population

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In maize, the Robertson's *Mutator* (*Mu*) as a multi-copy transposon family has been selected for saturation mutagenesis and allowed for the production of myriads of novel mutant alleles in the Maize-Targeted-Mutagenesis (MTM) collection. However, the potential value of this collection has not been properly realized due to the lack of mapped *Mu*-insertion alleles. We have sought to develop a method for efficient and large-scale FST identification in MTM lines, using a modified ligation-mediated GenomeWalker protocol coupled with high-throughput Illumina DNA sequencing technology. Here, we show that the position of newly transmitted germinal insertions in the genome can be identified on a large scale. We found that more than 100 *Mu* elements per plant were identified as germinally-transmitted insertions. Target sites flanking each MTM *Mu* insertion were detected over all 10 maize chromosomes but were distributed into hypomethylated genic regions and avoided highly-hypermethylated peri-centromeric regions. Furthermore, the methylation level of flanking regions where *Mu* targeted is even lower than hypomethylated exons in symmetric CG and CHG contexts. We detected two parental insertions for each germinal insertion in each plant, which is higher than is expected. Almost half of *Mu* target sites are inserted within 1 kb upstream of the start codon, suggesting that immobile parental insertions likely include Pack-MULEs that are preferentially located within 500 bp upstream from 5' termini of genes. We sequenced 96 DNA pools from one grid (48 X48) containing 2,304 plants, and further bioinformatic analysis will create a sequence-indexed MTM Mu FST database that will contain about 300,000 *Mu* insertion sites.

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P46

Leveraging non-targeted metabolite profiling via statistical genomics

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One of the challenges of systems biology is to integrate multiple sources of data in order to build a cohesive view of the system of study. Here we describe the mass spectrometry based profiling of maize kernels, a model system for genomic studies and a cornerstone of the agroeconomy. Using a network analysis, we can include 97.5% of the 8,710 features detected from 210 varieties into a single framework. More conservatively, 47.1% of compounds detected can be organized into a network with 48 distinct modules. Eigenvalues were calculated for each module and then used as inputs for genome-wide association studies. Nineteen modules returned significant results, illustrating the genetic control of biochemical networks within the maize kernel. Our approach leverages the correlations between the genome and metabolome to mutually enhance their annotation and thus enable biological interpretation. This method is applicable to any organism with sufficient bioinformatic resources.

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P47

Naturally occurring insertions in rice typically create tandem or local duplications that lack a signature of replication slippage

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The insertion of DNA into a genome can result in gene movement, the disruption of colinearity, and the duplication and dispersal of regulatory sequences. Such events may in turn lead to adaptation and speciation. A deeper understanding of insertion mechanisms will inform methods of genetic engineering and plant transformation. Exploiting structural variations in numerous rice accessions, we have inferred and analyzed intermediate length insertions in plants. Our findings indicate that, as observed in humans, tandem duplications are the dominant form of >10 bp insertions, although short duplications from ectopic donors account for a sizable fraction of insertions in plants. Though replication slippage is a plausible explanation for tandem duplications, the end homology required in such a model is often non-existent and rarely exceeds 5 bp. Yet, end homology is commonly longer than expected by chance. Such findings lead us to favor a model of synchronous base excision repair followed by non-homologous end-joining. Still unexplained are local duplications with intervening non-duplicated DNA sequence. Though current mechanistic models of double-strand break (DSB) repair are strained to predict such insertion scenarios, empirical results from induced DSB experiments exhibit a similar outcome. We present data on rice, maize, sorghum, and pearl millet to support this conclusion.

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P48

Parallel proteomic and phosphoproteomic analyses define proteotypes of successive stages of maize leaf development

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Maize leaves display a developmental gradient from base to the tip, which we exploited to compare protein and phosphoprotein abundance as developing maize leaves undergo successive phases of growth. Zone 1, at the leaf base, contains immature cells undergoing symmetric proliferative division; Zone 2 contains differentiating cells undergoing asymmetric division and early expansion; and Zone 3 contains post-mitotic, rapidly expanding cells. We also analyzed the blade of a mature leaf. Across four biological replicates, we identified over 81,000 peptides representing at least 12,000 unmodified proteins, and 11,000 phosphopeptides representing at least 3500 phosphorylated proteins. Global analyses reveal a core set of proteins common to all four leaf segments, but also reveal a set of proteins entirely unique to mature leaves, and another set shared only amongst the three growing zones. Strikingly, only a small portion of the proteome differs between the three young growing zones, but comparison of the phosphoproteomes reveals many proteins uniquely phosphorylated in a single zone. This suggests that growth and differentiation is post-translationally controlled. Deeper analyses of the proteomic and phosphoproteomic profiles of cell wall-related and hormone-related proteins provide further insight into phases of maize leaf growth. Novel phosphorylation sites were observed in many of these cell wall- and hormone-related proteins, including several whose abundance do not correlate with protein abundance, potentially signifying novel regulatory sites. Amongst these sites is a single phosphorylated residue in ZmPIN1 that, unlike the other observed ZmPIN1 phospho-sites, does not correlate with ZmPIN1 abundance but does correlate with changes in PIN1 polarization in the epidermis. This study not only provides insight into the regulatory processes defining maize leaf growth, but also provides a data-rich community resource of protein abundance and phosphorylation status.

Funding acknowledgement: National Science Foundation (NSF)

P49

Pattern and distribution of deleterious mutations in maize inbred lines

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Amino acid changes caused by nonsynonymous single nucleotide polymorphisms may negatively impact protein function. These deleterious polymorphisms are in general removed by selection but may be fixed by drift or by hitchhiking with advantageous loci. To better understand the pattern of deleterious mutations in maize inbred lines, a whole genome scan for potentially deleterious amino acid polymorphisms was carried out. Using 400,000 genotyping by sequencing SNPs, we quantified deleterious mutations in a panel of 282 maize inbred lines representative of the main genetic groups. As expected from theory, nonsynonymous polymorphisms were generally at lower frequencies than synonymous polymorphisms, and a large proportion of nonsynonymous changes were predicted to be deleterious. Finally, we described associations between a number of quantitative traits and loci with deleterious polymorphisms.

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P50

Patterns of computed conserved noncoding sequence loss following the paleopolyploidies in the maize and Brassica lineages

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The maize paleotetraploidy and the Brassica rapa paleohexaploidy generated homeologs diverged at Ks 0.15 and 0.37, respectively. The intergenic DNA of these polyploids have not been assembled to the same quality as in their outgroups, sorghum and arabidopsis. An efficient way to find CNSs in these polyploids is to map them over from grass CNSs in sorghum or from crucifer CNSs in arabidopsis. We coded a simple pipeline to map over CNSs from the more reliable, more ancestral outgroup onto the polyploid using blastn and, at the same time, apply global alignment with sequence simulation controls to discover remnants of sequence that may identify those rare CNSs lost, but not by deletion. Deletion by intrachromosomal recombination is the preferred mechanism of duplicate gene loss, as reviewed (Freeling et al. 2012. Cur. Opinion Plant Biol) and also duplicate CNS loss in Brassica rapa (Subramaniam et al. 2013, submitted). Using the Map Over Pipeline (unpublished), we focused on retained gene pairs that fractionated one or more of their CNSs, either by subfunctionalization or nonfunctionalization. For Brassica rapa, 15% of CNSs are lost (deleted); we will report on what we have learned by following the pattern of these deletions. Results include 1) Some CNSs remain as orphans after their supposed gene is deleted. 2) The frequency of subfunctionalization vs. nonfunctionalization increases as CNS content increases. 3) Nonfunctionalized cis-acting space has about an 80% chance of being from the not-dominant subgenome. 4) Runs of nonfuctionalization are far more common than expected by chance alone, so we conclude that some CNS groups encode a single cis-acting function. We are trying to repeat our results in maize, at least on gene pairs with well-assembled noncoding space. We have begun the process of correlating patterns of CNS loss with changes in gene expression.

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P51

Quantitative Assessment of Complex Visual Phenotypes in Maize Lesion Mutants

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Accurate definition of a phenotype is often the first step in understanding the genetic mechanisms responsible. Complex visual phenotypes are especially difficult given the numerous, and often nebulous, traits which may be used to describe them. In *Zea mays*, the lesion mimic mutants are a class of mutants which spontaneously develop areas of dead tissue without injury or infection; these lesions may vary considerably in size, shape, color, and distribution, among other traits. Two mutants in particular, the *Les2* and *Les4* mutants, provide phenotypes which are visually distinct, making them good candidates for comparison. To do this, we have collected numerous photographs of mutant leaves and are developing algorithms to automatically segment and analyze their lesions. Once analyzed, the data, which may be real or categorical, can be plotted in a high dimensional phenotypic space. This provides the means for a more accurate and quantitative description of the *Les2* and *Les4* phenotypes, and when compared with genetic sequence data, may provide clues to the pathways responsible.

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P52

Reshuffling of Genic Variation via Meiotic Recombination Generates Novel Gene Expression Patterns

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The shuffling of DNA polymorphisms among haplotypes via meiotic recombination can create novel alleles. We analyzed Genotyping-By-RNA-Sequencing data of 105 IBM recombinant inbred lines (LI et al. 2013) to examine genome-wide patterns of meiotic recombination and study how intragenic recombination affects gene expression. In total, 7,574 crossovers and 922 non-crossovers (putative gene conversions) were observed. As expected based on previous reports, crossovers were more common along chromosomal arms and less common in pericentromeric regions and at the ends of chromosome. We identified 793 recombinant alleles of 591 distinct genes. Consistent with previous studies on single genes, intragenic recombination events occurred preferentially at the 5' ends of genes. 39% of all intragenic crossovers occurred within 107 "hotspot genes", each of which experienced at least two independent intragenic crossovers. Characterization of these hotspot genes indicates that they exhibit low levels of heterochromatin-related epigenetic marks. Importantly, we validated the hypothesis that meiotic recombination creates functional variation by identifying recombinant alleles that exhibit non-parental expression patterns. Such novel alleles represent a source of genetic novelty for evolution and breeding.

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P53

RNA-Seq Analysis of Maize Gametophytic Transcriptomes

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Both male and female gametophytes play central roles in sexual plant reproduction. A hallmark of the plant life cycle is that gene expression is required in the haploid gametophytes. Consequently, many mutant phenotypes are expressed in this phase, affecting transmission of the mutant allele. For large-scale identification of gametophyte-expressed genes, we have performed RNA-Seq from embryo sacs, comparator ovules, mature pollen, and seedlings. The transcriptomes of the two gametophytes appear fairly distinct, as only ~5% of the loci enriched in at least one of the gametophytic samples are enriched in both. Genome wide analysis of the available *Ds* and *Uniform Mu* insertion mutant collections shows a statistically significant deficit in insertions in gametophyte-expressed genes and validates RNA-Seq as a method for identifying gametophyte-essential genes. Transcriptome analysis reveals that repetitive elements of many classes are highly expressed in the female gametophyte. Transcript assembly against the maize genome identifies a large number of putative novel gene models expressed in these tissues. Gene ontology analysis demonstrates enrichment for small signaling proteins and transcription in the female gametophyte and cytoskeleton and cell wall modification in the male gametophyte. Validation of a subset of gametophyte-expressed genes is being performed using *in situ* hybridization and mutant analysis.

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P54

The iPlant Collaborative's DNA Subway: An Easy-to-Use Tool for Community Annotation of Maize and Other Genomes

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Current efforts to improve the maize B73 reference genome involve not only improvements to the assembly but also improvements to structural gene annotation. Optimized automated structural gene annotation workflows and distributed approaches to community gene curation are required. Work is ongoing to adapt existing tools such as MAKER and the extensible Genome Data Broker (xGDB) for structural gene annotation. yrGate and Web Apollo are being evaluated for use in community-based annotation and curation for B73 and other genomes. Web Apollo is a distributed web-based application based on the JBrowse genome browser. Web Apollo's predecessor, Apollo, was embedded as a curation tool in the iPlant Collaborative's DNA Subway (www.dnasubway.org), which was originally developed as easy-to-use gene annotation software. DNA Subway packages complex bioinformatics tools and data into an intuitive user interface. Four analysis paths or "subway lines" are available: the Red Line for gene annotation; the Yellow Line to survey plant genomes for transposon families; the Blue Line for DNA barcoding and phylogenetics; and the Green Line for RNA-Seq analysis. The Red Line supports the evaluation of gene models, generated by several embedded ab initio gene prediction tools, using external evidence, such as ESTs, BLAST results, etc.

DNA Subway is still under active development. Key new features of the Red Line include: 1) integration of Web Apollo; 2) integration of JBrowse for contextual viewing of annotations; 3) support for importing annotations generated by external workflows; 4) integration with the Green Line to support the use of RNA-Seq data as a class of evidence for evaluating transcript structure; and 5) Support for exporting annotations to third-party repositories, such as maizeGDB. DNA Subway is a good candidate for distributed, community gene annotation, as it has the ability to perform RNA-Seq analysis and use the resulting annotated transcripts as a class of evidence for evaluating gene models within the DNA subway, the ability to import externally-generated annotations, and is easy to use. DNA Subway is therefore well aligned with efforts to improve the maize B73 reference genome assembly and other upcoming assemblies.

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P55

The Maize Genome Project, an Update

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Work continues on refining the assembly and annotation of the B73 reference sequence. The RefGen_v2 assembly and annotations are hosted on Gramene and have been deposited in GenBank along with contigs assembled from Roche/454 sequencing of a whole genome shotgun library. RefGen_v3 incorporates some of these contigs in order to increase coverage of missing gene space. Contigs were selected based on alignment to FLcDNA sequences and inserted into gaps in the RefGen_v2 assembly guided by a genetic map and synteny to rice and sorghum. Approximately 500 genes were added or improved with new annotation. A number of unplaced physical map contigs were also anchored based on these maps. The RefGen_v3 assembly will be released following acceptance by GenBank. At that time, maizesequence.org will be deprecated and requests will be redirected to gramene.org. Work on RefGen_v4 is also underway. In order to improve the maize genome sequence we are adding additional depth to the existing capillary sequence using the Illumina 2000. We are resequencing 17,173 BACs that comprised the minimal BAC tiling path from the original B73 sequencing project by pooling the BACs in pools of 96, and generating Illumina data to greater than 150X coverage per clone. This increased depth, density of read pairs, and differential bias compared to capillary sequencing will serve as an excellent resource to further improve the maize sequence. These data, once completed, will be made available through public databases prior to completion of the RefGen_v4 assembly. This work was funded by the NSF/DOE/USDA "Sequencing The Maize Genome" project (NSF #0527192 and #0910642).

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P56

The molecular basis of adaptation to highland climates in domesticated maize

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After its domestication in the Balsas River Valley in the Mexico lowlands, maize spread quickly across the Americas. Adaptation to highland climates in Mexico and South America occurred independently from locally-adapted lowland maize. Here, we show the result of a genome scan to identify loci involved in parallel adaptation to highland climates. To do this, we generated two large SNP data sets of 94 maize landraces, sampled from Mexico and South America. We first inferred the demographic history of these lowland and highland populations. We used the inferred demographic history to then perform a genome scan to identify highly differentiated loci that may have contributed to highland adaptation to highland climates. The majority of loci identified show evidence of selection in highland habitats from standing variation, yet we see little evidence of parallel adaptation. We discuss the significance of these results in the context of the molecular basis of adaptation to new environments.

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P57

The reference genome based genotyping-by-sequencing (GBS) bioinformatics pipeline in TASSEL4

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Genotyping by sequencing (GBS) is a reduced representation, next generation sequencing approach that provides a robust and cost-effective means to genotype large numbers of individuals at high density, by targeting sequence adjacent to restriction enzyme cut sites. We have built a reference genome based bioinformatics pipeline for the efficient analysis of the large volume of raw sequence data that can be produced by GBS. Key features of this pipeline, which is part of the TASSEL4 standalone, are speed, scalability, and high capacity. The high capacity of the pipeline is afforded by the use of the HDF5 data storage format. The pipeline was constructed with the complex genome of maize in mind, and thus uses methods to detect and remove error prone SNPs that are suitable for other plant species with complex (but diploid) genomes. SNP calling and filtering are performed at the species (or genus) level in a "discovery" phase intended to involve all samples sequenced to date. Rapid analysis of newly available GBS sequence can be achieved via the "production" phase, which takes advantage of the knowledge gained from the discovery phase to quickly call known variants. As part of TASSEL, the pipeline is written in open source code available from SourceForge. The TASSEL4 standalone, along with detailed documentation of the GBS pipeline itself, is available at www.maizegenetics.net/tassel/.

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P58

Transcriptional Modules in Maize Development

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Transcriptional control is an important aspect of plant development, and genes which are transcriptionally coordinated often participate in related biological processes. We describe a genome-wide overview of transcriptional circuits that control development in *Zea mays* (maize). We examined transcript abundance data at 50 developmental stages, from embryogenesis to senescence, for 34,876 gene models and classified genes into 24 robust coexpression modules. Genes within modules with tissue-specificity are enriched for GO terms. For a number of modules, key genes involved in transcriptional control have expression profiles that mimic the expression profiles of module genes, although the expression of transcriptional control genes are not unusually representative of module gene expression. Known regulatory motifs are enriched in several modules, and we present novel promoter motifs. Based on genes' expression profiles and module memberships, we predict the function of a number of uncharacterized genes. Finally, of the 13 network modules with more than 200 genes, three contain genes that are notably clustered ($p<0.05$) within the genome. This work, based on a carefully selected set of all major tissues representing diverse stages of maize development, demonstrates the remarkable power of transcript-level coexpression networks to identify underlying biological processes and their molecular components.

P59

Transcriptomic diversity among maize inbreds

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To globally identify expressed PAV genes the transcriptomes of 5 tissues from the 27 NAM founders were deeply sequenced. We have previously reported on the identification and characterization of these PAVs (Ying et al, MGC, 2011). This RNA-Seq dataset has been further analyzed to systematically investigate other types of transcriptomic variation. Consistent with the hypothesis that variation in gene regulation contributes to phenotypic diversity, the genes that exhibit the most variable expression across genotypes are enriched in regulatory functions. In contrast, genes that exhibit highly variable expression across tissues are not enriched in regulatory functions. Approximately 5 million SNPs and small InDels were discovered in this RNA-Seq dataset. 65% of these variants are located in the transcribed regions of FGS genes, making this dataset a rich source of genic variation. Analysis of these genic SNPs allowed us to determine that the minimum numbers of haplotypes per gene in the NAM founders are substantially higher the previous estimates. Consistent with the complementation model of heterosis thousands of FGS genes are affected by Large Effect SNPs (LES), such a premature termination codons, splice site variants etc. Probably as a consequence of enrichment for genic SNPs in our data set our estimate for the number of FGS genes affected by LES is approximately an order of magnitude higher than earlier estimates. ~20% of these LES are present in only one of the 27 analyzed haplotypes. Consistent with the subgenome hypothesis (Schnable J., et al, PNAS, 2011) retained homoeologous genes of maize subgenome 2 are much more likely to harbor LES than those from subgenome 1. An additional ~15% of LES are present in all haplotypes except B73; at least some of the affected FGS genes are probably mis-annotated. These RNA-Seq data provide evidence for the expression across genotypes and tissues of thousands of WGS genes that are not included in the FGS. Finally, using stringent cut-offs we identified thousands of novel transcribed regions that are not included in the WGS.

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P60

UniVIO: a multiple omics database with hormonome and transcriptome data from rice

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Plant hormones play important roles as signaling molecules in the regulation of growth and development by controlling the expression of downstream genes. Since the hormone signaling system represents a complex network involving functional crosstalk through the mutual regulation of signaling and metabolism, a comprehensive and integrative analysis of plant hormone concentrations and gene expression is important for a deeper understanding of hormone actions. We have developed a database named Uniformed Viewer for Integrated Omics (UniVIO: http://univio.psc.riken.jp/), which displays hormone-metabolome (hormonome) and transcriptome data in a single formatted (uniformed) heat map. At the present time, hormonome and transcriptome data obtained from 14 organ parts of rice plants at the reproductive stage and seedling shoots of 3 gibberellin signaling mutants are included in the database. The hormone concentration and gene expression data can be searched by substance name, probe ID, gene locus ID, or gene description. A correlation search function has been implemented to enable users to obtain information of correlated substance accumulation and gene expression. In the correlation search, calculation method, range of correlation coefficient, and plant samples can be selected freely.

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P61

A comparison of MiRNA targets involved in embryo development in maize, sorghum, rice and barley

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A recent transcriptomic analysis of Maize(Shen et al) and rice has revealed several genes to be differentially expressed. These differentially expressed genes (DEGs) are highly conserved both in expression level and in sequence. We sought to find out if they are just as conserved across several cereals such as barley and sorghum. We also examined enhancers and miRNAs associated with these genes. We aligned the miRNAs to see if they were as conserved as their targets. These genes are involved in carbohydrate metabolism and transport related.

Some of the genes we examined are:

Beta-glucosidase, chloroplastic Beta-glucosidase, chloroplastic
Alpha-amylase isozyme 3B, Beta-glucosidase 22 Aquaporin PIP1-5 Beta-glucosidase 31 Glycogenin-2
Glyceraldehyde-3-phosphate dehydrogenase B, chloroplastic, Beta-fructofuranosidase 1
GDP-mannose 4,6 dehydratase 2 Beta-glucosidase, chloroplastic , Uncharacterized protein At2g34460, chloroplastic Aldose 1-epimerase

We also examined some introns to see if these genes were good candidates for splice variants, thus providing for a relaxation of the levels of conservation of mi-RNA targets. The genes shown above were down-regulated, as was to be expected, and we examined the most up-regulated genes for mi-RNA targets as well. While maize and sorghum show similarities(Zhang et al), rice occupies a different niche. We concluded that while there is a good measure of conservation across all these genera, mi-RNAs themselves may not be as conserved, making predictions a little less reliable.

P62

A mutation in *DNA polymerase α* co-segregates with a *defective kernel* (*dek*) phenotype in maize

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The maize seed provides one of the world's most important sustainable sources of food, feed and industrial raw material. Understanding the molecular genetics of seed development is of paramount importance for future crop improvement. In eukaryotes, three DNA polymerases (α, δ, and ε) have been identified as the main enzymes to replicate the nuclear genome. DNA polymerase α forms a functional complex with primase to synthesize RNA primers for DNA Polymerase δ and DNA Polymerase ε to elongate the lagging and leading strands, respectively. Based on its fundamental role in DNA replication, DNA polymerase α is expected to be an essential gene for all cells. Surprisingly, we found a *DNA polymerase α* mutant co-segregates with a *defective kernel* (*dek*) phenotype and is still capable of developing significant endosperm and embryo tissue. The *dek* was isolated from the UniformMu transposon tagging population and mapped to a 35 cM interval on the long arm of chromosome 3 using Bulk Segregant Analysis (BSA) and quantitative SNP markers. Further genetic mapping with 95 mutant kernels from a Mo17 x UniformMu F2 population narrowed the mutation to a 7 Mbp interval. By co-localizing the genetic map positions with transposon flanking sequence tags (FSTs) from the *dek* mutant, we identified a candidate insertion in the first exon of DNA polymerase α. This insertion co-segregates with the seed mutant phenotype suggesting that mutations in DNA polymerase α allow some DNA replication. Characterization of this mutant is expected to reveal the mechanism by which cell cycle and DNA replication determine seed size.

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P63

A new floury endosperm mutant with a mutated z1A 19kD zein gene

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In this study, we have identified a new semi-dominant floury endosperm mutant in maize. The mutant seed has typical opaque/floury endosperm with changed zein composition and increased lysine content. The mutant endosperm has misshapen zein protein bodies and increased level of ER luminal binding protein (BiP) during seed development. The mutant gene was mapped to the short arm of maize chromosome 4, overlapped with z1A 19kD α-zein gene cluster. Using immunoblotting with 19kD zein antibodies, we identified an extra 19kD α-zein band in mutant endosperm proteins. Sequencing analysis of z1A cDNAs from immature mutant endosperm had identified a mutated19-kD α-zein gene with a point mutation at the cleavage site of signal peptide. This may led to an un-cleavable signal peptide during protein synthesis and processing, similar to maize floury2 mutant gene. This mutated cDNA was expressed in E.coli, and the resulting protein product migrated at the same position as the extra 19kD α-zein band identified from the mutant endosperm. We have been creating transgenic maize plants expressing this cDNA to confirm weather this mutated gene is responsible for the mutant phenotype.

Funding acknowledgement: Chinese Ministry of Science and Technology

P64

A new transcriptional factor for 19kD α-zein z1A gene family

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The α-zein gene family encodes the most prominent storage proteins in maize seeds, and is constituted by 22kD zein (z1C) and three 19kD zein (z1A, z1B and z1D) gene sub-families. It is known that Opaque2 is the specific transcriptional factor (TF) for 22kD α-zein sub-family. However, the specific TF(s) for the three 19kD α-zein sub-families is remained unclear. In order to identify novel TF(s) for 19kDa α-zein genes, we carried out a Yeast One-Hybrid screening using z1A gene promoter against a maize seed cDNA fusion library. A novel C3HC4 Zinc Finger protein was identified that could specifically bind to z1A core promoter based on EMSA. Moreover, its nuclear localization and transcriptional activation properties suggested this protein was a transcriptional activator. Indeed, this protein was able to trans-activate z1A promoter in a transient reporter system within living plant cells. Promoter deletion and point mutation analysis revealed that this protein recognized the GT-motif in the z1A core promoter, hence was designated as GT-motif binding factor (GBF). Interestingly, Opaque2 was also found to be able to recognize an O2 binding motif, 10bp apart from the GT-motif, and trans-activate the z1A promoter. Moreover, GBF and O2 could interaction to each other in vitro and in vivo. When both factors were co-expressed, the transcriptional activation to the z1A promoter was greatly enhanced, suggesting a synergistic interaction between GBF and O2 on z1A gene transcriptional regulation.

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P65

A Novel Functional Genomics Method for Identification of Opaque2 Modifier Genes and Other Genes Involved in Maize Seed Development

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Quality Protein Maize (QPM) is a hard kernel version of the high-lysine *opaque2* mutant. Mapping experiments have identified major QTLs for kernel modification. With the exception of 27-kD gamma-zein within the chromosome 7 QTL, the nature of o2 modifier genes are unknown. We are using a gamma-irradiation mutagenesis approach to investigate these QTL regions and potentially identify other regions containing o2 modifier genes and general seed development genes. In this study, we mutagenized and propagated 2000 kernels of K0326Y QPM. 293 M2 ears were recovered and M2 screens identified a number of opaque revertants. In the M3 generation, we have identified 24 heritable opaque revertants as well as other classes of seed phenotypes and whole plant developmental and/or biochemical mutants. Zein protein analyses showed several significant classes of zein alteration, including severe reduction of residual 19-kD or 22-kD alpha-zein and a 27-kD gamma-zein null mutant. Such mutants give information on zein function in vitreous endosperm formation and suggest novel avenues for production of non-transgenic, non-pleiotropic, low-zein, high-lysine breeding stocks. We are using Illumina sequencing of exome-enriched genomic DNA from pools of mutants to identify deleted exons. As proof of concept, this approach has shown the 27-kD gamma zein null mutant results from a large deletion covering this gene as well as the nearby 50-kD gamma zein gene. RNA-seq is also being used to relate expression abolition to genomic deletions which will assist in the association of mutations with specific phenotypes.

Funding acknowledgement: Department of Agronomy and Horticulture

P66

Adult Plant Resistance in the Maize-CCR1 pathosystem: A Biochemical Basis

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Cochliobolus carbonum race 1 (CCR1) is one of the most destructive fungal pathogens of maize, causing lethal leaf blight and ear mold. A key virulence factor involved is HC-toxin, a cyclic tetrapeptide with broad-spectrum histone deacetylase activity. To counter HC-toxin, the maize *Hm1* gene 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. To test the biochemistry underlying this APR, we have expressed the HM1, HM1A, and HM2 enzymes *in vitro* and have confirmed enzymatic activity. Concurrently, we are using site-directed mutagenesis to alter specific residues predicted to be essential for proper enzymatic activity. We are currently determining the kinetics for these mutant HCTRs to elucidate whether their enzymatic activities are compromised compared to HM1 at lower levels of the NADPH cofactor. To further investigate the potential role of NADPH in defining APR, we are also quantifying temporal and developmental changes of *in planta* NADPH levels.

P67

Analysis of candidate genes for multiple-stress responses

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Hormones are well known for their central role in regulating plant growth and development, but their role in response to abiotic stress is less appreciated. Variations in hormone balance can alter stress-related gene expression responses in maize. We are specifically investigating the role of hormones in multiple-stress-response pathways for plant traits. It has been previously established that the genetic pathways, the important loci, employed during multiple-stress responses are different from those used during single-stress responses. Hormone-related maize gene loci will be compared to multiple-stress quantitative trait loci, and maize genes shown to be important for both hormone activities and multiple-stress pathways identified. This will be done by comparing quantitative trait loci with lists of hormone-associated genes from the literature. The results will be validated in field experiments to observe the effect of mutants in the candidate genes in combined-stress environments including fungal pathogen, drought, and nitrogen stress.

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P68

Analysis of the role of the ZmMYBGA1 transcription factor in the regulation of amylase genes in maize

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In rice, MYBGA is known to regulate the alpha-amylase genes that degrade starch in the seed during germination. MYBGA also activates the cell death of the tapetum, which assists in the development of the anther and MYBGA is inhibited by a family of proteins called DELLA proteins. We predict that the ZmMYBGA1 protein also functions in seed germination and tapetal development. We first used the rice MYBGA TF to identify the homologs in Zea mays (GRMZM2G028054 on Chr8 and GRMZM2G139688 on chr3) and related grass species and we performed phylogenetic comparison of the identified MYB-related genes and proteins encoded therein (gene structures and protein motifs). A full length ZmMYBGA gene was cloned and the DNA binding domain and unique regions were subcloned into a Gateway entry vector. These constructs were recombined into and overexpressed protein in E. coli for DNA binding studies. We also identified and examined candidate target amylase genes using bioinformatics to begin to identify the regulatory network in which ZmMYBGA operates. We identified at least one amylase gene that is expressed specifically in germinating seeds and we are cloning the promoter genes for expression studies in yeast. Finally, we used the Bio-Array Resource efp browser (http://bar.utoronto.ca) to examine expression patterns of MYBGA1 and candidate target genes in various corn tissues. This project was funded by in part by grant NSF DBI-0701405, IOS-1125620, and by the Ohio Plant Biotechnology Consortium

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P69

Assembling the Puzzle: A Closer Look at the Maize Phenolic Pathway Regulators

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The phenolic biosynthesis pathway in maize produces a number of different compounds, such as lignin and anthocyanins, depending on what regulating proteins are active. In an effort to better understand this regulation, we have chosen 28 maize transcription factors believed to play a direct role as activators or repressors of genes in the pathway, or which have been recently shown to be direct targets of the main regulators. Our goal is to conduct a comprehensive yeast two-hybrid screen using these 28 transcription factors as baits, and a newly-constructed prey library consisting of nearly 3500 maize transcription factors. Here we show our preliminary results of transcriptional self-activation and/or homodimerization of the bait proteins. Those positive for either of these interactions are further analyzed by site-directed mutagenesis to repress these activities. Information gained from this preliminary work, as well as the final Y2H screen, will enable researchers to assemble a complete picture of the regulatory network involved in the phenolic biosynthesis pathway, as well as access an invaluable collection of transcription factors for further study.

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P70

Auxin Evo-Devo: Genetic and genomic approaches to understanding the role of auxin in shoot development

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Auxin regulates nearly every aspect of plant growth and development. A better understanding of auxin's function is therefore fundamentally important to basic plant biology and crop improvement. Previous research has demonstrated both conservation and diversification of auxin's role in maize and *Arabidopsis*. This project will further our understanding of how auxin regulates shoot development, emphasizing maize shoot organogenesis.

To identify additional genes functioning in auxin-mediated organogenesis, we are characterizing 147 maize mutants with distinctive vegetative and reproductive developmental defects. Together with previously characterized mutants, we have mapped 71 mutants to 40 locations in the maize genome. Eight of these loci had been cloned previously, and an additional six genes have been cloned on this project. Many of these genes encode proteins required for auxin biosynthesis, transport and response. Preliminary phylogenetic analyses of 15 gene families have illustrated complex relationships amongst monocot and eudicot clades. Reverse genetic efforts guided by these phylogenetic relationships have confirmed 17 insertions in 11 genes. Further phylogenetic, functional and comparative expression analyses will test the conservation and diversification of auxin action mechanisms in all flowering plants.

www.AuxinEvoDevo.org

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P71

Biochemical Analysis of Kernel Texture and Protein Quality in Maize Endosperm

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Quality Protein Maize (QPM) is a hard endosperm version of the high lysine *opaque2* (*o2*) mutant but aside from 27-kDa gamma zein, the genes involved in modification of the soft *o2* endosperm are unknown. Pyrophosphate (PPi)-dependent fructose 6-phosphate 1-phosphotransferase (PFP) catalyzes the ATP-independent conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate in glycolysis. We previously found a large increase in transcript and protein levels of the alpha regulatory subunit of PFP (PFPα) in QPM endosperm. In vitro enzymes assays show a significant increase in forward PFP activity in developing endosperm extracts of QPM relative to wild type and *o2*. PFPα is co-induced with heat shock proteins (Hsps) in maize roots in response to heat, cold and ER stresses. The elevated expression levels of a number of ATP-requiring Hsps in *o2* endosperm may result in an ATP crisis which could contribute to the *o2* phenotype. In QPM, this general Hsp response is ameliorated. We propose there is an energy currency switch from ATP to PPi in the developing endosperm of QPM that correlates with increased metabolic flux through glycolysis that result in increased ATP production allowing for normal endosperm development. Furthermore, O2 regulated pyruvate Pi dikinase (cyPPDK1) gene is reduced in expression and activity in *o2* leading to decreased glycolytic flux and possibly reduced ATP production, which the PFP activity increase may serve to compensate. In contrast, the O2 independent cyPPDK2 gene seems to have a compensatory increase which may also have a role in cellular stress adaptation that is specific to QPM.

P72

Biogenesis of Protein Bodies in Maize Endosperm

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The α-, γ-, β- and δ- zeins are specifically expressed in maize endosperm from 10-12 DAP. The γ- and β-zeins are thought to prime protein body initiation and through cross-linking to play a role in stabilizing the peripheral shell of mature PBs, thus encapsulating the central core of hydrophobic α-zeins. RNAi reduction of these zeins causes amorphous PB shapes. Combination of α22RNAi, γRNAi and βRNAi caused dramatic morphological changes in PB structure and clumping of small PBs. We used immuno-TEM to examine the spatial location zeins and found that whereas the α19 zeins are located in the PB inner core in WT and α22RNAi lines, they are displaced to the PB boundaries in the double γ/βRNAi and in the α22/γ/βRNAi triple lines, indicating that γ- β- and α22-zeins together are essential for delimiting the domains of PBs. Since proper location of α22-zeins depends on FL1, we stacked the *fl1* mutant and γ/βRNAi to create second triple mutant, in which PBs were extensively clumped as in the first triple mutant of α22RNAi, γRNAi and βRNAi, indicating that normal expression and correct deposition of α22-zeins are critical for PB delimitation in the absence of γ- and β-zeins. The *fl1-ref* allele accumulates some FL1 protein but the point mutation delayed its electrophoretic migration, which might affect FL1 function. Also, an apparent FL1 dimer which exists in all genotypes is much more abundant in non-transgenic and transgenic lines in the *fl1/fl1* background. By comparison of total denatured protein extracts with native soluble extracts, we show that more FL1 partitions into the soluble fraction in all zein RNAi lines than wild type. This is confirmed using sucrose density gradients, which show more FL1 partitioning into the less dense cER fraction than the protein body ER fraction in all zein RNAi lines.

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P73

Biomolecular characterization of ancient maize

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Maize has experienced major phenotypic and genomic modifications over the course of its domestication in the last 10,000 years. These changes have been studied intensely through genetic data from modern material, but little work has been carried out on ancient specimens.

However, by testing archaeological specimens using state-of-the-art biomolecular techniques, it is possible to get a snapshot of the genetic and proteomic state of maize plants thousands of years ago. The underlying genetic changes can thereby be studied directly, making it possible to gain new insights into the process of domestication. Here we present our research methodology used to characterize ancient maize specimens with high-throughput DNA, RNA, and protein sequencing. The pipeline involves the extraction of biomolecules, DNA library building for next generation sequencing, PCR amplification, targeted enrichment via hybridization capture, high-throughput sequencing, and several controls to enable identification of genuine ancient molecules, both during laboratory processing and data analysis.

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P74

C-partitioning, defective kernels (deks), and pentatricopeptide-repeat proteins (PPRs)

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Carbon partitioning has crucial roles in regulating plant growth and development. Fixed carbon typically moves as sucrose from leaf cells to phloem, and is then partitioned to sink tissues throughout the plant. Our goal was to identify genes affecting this process. Towards this end, we used the transposon-mutagenic UniformMu population to select mutations in genes of interest, and also to select mutant phenotypes potentially related to altered carbon partitioning. We hypothesized that some of these forward- and reverse-genetic targets would show an informative co-segregation between genotype and phenotype. Genes for analysis were identified using the UniformMu database (via MaizeGDB.org) for map sites and flanking sequences of *Mu*-insertions. Gene-specific primers were designed for each insertion, and 15 to 25 progeny were PCR-genotyped for each family of interest. Co-segregation was then tested between phenotype and presence or absence of the *Mu*-insertions selected. A positive correlation was observed between a defective kernel (dek) mutant and Mu disruption of a *pentatricopeptide-repeat-protein (PPR)* gene. A similar cosegregation of genotype and phenotype was also observed for a second allele of this PPR gene, and additional analyses are in progress. An association between this insertion and its phenotype brings us one step closer to the identifying possible roles of this gene, and ultimately, a better understanding of plant growth.

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P75

Canalization at the *r1* Locus in Maize

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The *r1* locus, on the long arm of chromosome *10*, contains transcriptional activator genes responsible for the initiation of tissue-specific anthocyanin production. Although *r1* genes have been extensively studied since the 1950's, little is known about how these genes are buffered against perturbations in expression (canalization). Here we report on canalization of derivatives of *R-sc*, a haplotype containing one *Sc (self-colored)* and two *Nc (near-colorless)* genes that cause dark purple/black seeds and near colorless-lightly mottled seeds, respectively.

In 1961 K. McWhirter, in a screen for mutations of *sc* alleles, reported recovery of an atypical cluster of brown kernels on an ear of black seed. The kernel phenotype was found to be semi-heritable and selectable over several generations, resulting in a series of epialleles of light to dark kernels. The basis for the variable phenotype maps at or very near the *r1* locus. In order to determine the bases for the loss of canalization, EMS was used to induce mutations of the lightest epialleles resulting in increased kernel pigmentation and potential reversion of the original canalized dark seed phenotype. Of the 30 putative mutants identified, seventeen have been mapped relative to the *r1* locus. Twenty-one of the 30 have been intercrossed to determine complementation. Based on preliminary data, the new mutants map at or near the *r1* locus and elsewhere in the genome. At least two genes have been found to be involved in the canalization of *R-sc*.

Further molecular tests are being used to identify the structure and methylation patterns of these mutants. Additional mapping tests will determine the location of the change (s) relative to the *r1* locus. These studies will provide information on gene expression, and begin to identify genes in the canalization at *r1*, and potentially other loci.

Funding acknowledgement: Virginia Commonwealth University

P76

Carbohydrate Analysis by Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS)

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Carbohydrates play important roles in biological systems. In plants, they are synthesized during photosynthesis and translocated to the non-photosynthetic sink tissues to sustain their growth and development. Progress in the understanding of these processes, however, primarily depends on our ability to accurately measure levels of individual carbohydrates. Current, most advanced techniques utilize GC or LC separation and mass spectrometer for detection. These methods have limiting resolving power to separate isobaric carbohydrates with similar structures an they are relatively time consuming preventing large scale studies. Alternative solution is the use of ion mobility separation-mass spectrometry (IMS-MS). This system was used to study the effect of ion adducts on the separation of isomeric trisaccharides and disaccharides. The ion mobility drift time was measured for each carbohydrate as each metal ion adduct. As expected, the size of the metal ion exerted a significant influence on the drift time of the adduct ions. For example, the drift time of adducts of maltotriose varied from 5.89 ms for the [M+Li]+ ion to 6.79 ms for the [M+Cs]+ ion. Differences in drift time were also noted for isomers coordinating the same metal ion. The results indicate that the collision cross sections of a particular carbohydrate usually increase with the radii of the coordinated metal ions. The method was validated by determination of the limit of detection (LOD), lower limit of quantification (LLOQ), and linearity range for maltotriose and raffinose. For both compounds, the LOD and LLOQ were established at 0.2 μM and 0.4 μM, respectively, with linearity ranging over more than two orders of magnitude. Our data suggests that IMS-MS analytical platform can be used for both efficient separation and accurate determination of carbohydrates. In addition, our method significantly increases sample throughput as a time required for analysis of 1 sample is less than 5 min.

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P77

Carbohydrate characterization of the *isa2-339* allele and its interaction with *su1-ref* in maize

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The *su1-ref* mutant, which results in an inactive isoamylase1 has been influential in determining the role of starch debranching enzymes in starch biosynthesis in the developing maize endosperm. *isa2-339* is a mutation that knocks out another starch debranching enzyme, isoamylase2. The protein products of the *Su1* and *Isa2* genes are known to have specific protein-protein interactions in developing endosperm. This experiment was conducted to determine if the visual kernel phenotype and carbohydrate traits of the double mutant of *su1-ref* and *isa2-339* were more or less severe than a *su1-ref* parent. Additionally, the phenotypic expression and carbohydrate profiles of *Su1 isa2-339* genotypes were observed to determine if this endosperm mutant could condition unique visual or biochemical endosperm traits. Visual selection of the progeny of a cross between a *su1-ref Isa2* and a *Su1 isa2-339* parent was used in the F_2 and F_3 generations to identify unique kernel phenotypes. Individual F_5 kernels were classified into specific phenotypes and subsequently genotyped to associate with visual kernel appearance and carbohydrate traits. Kernels homozygous for the *su1-ref* and *isa2-339* alleles contained significantly greater concentrations of total sugars and significantly lower concentrations of starch than the *su1-ref* parent. Kernels genotyped as homozygous *isa2-339* with a mutant, non-sugary kernel phenotype had significantly greater levels of phytoglycogen than the *isa2-339* parent. These data demonstrate that kernels with the *su1-ref isa2-339* can have a severe reduction in starch compared to *su1-ref* genotypes and the *isa2-339* allele can condition a unique kernel phenotype with a significant increase in phytoglycogen accumulation. This suggests that the ISA2 protein could interact with more than the SU1 protein and have additional biological functions in starch synthesis.

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P78

Carbohydrate Partitioning Defective1 functions in carbon partitioning and plant defense

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In maize, the transport of sucrose from the photosynthetic leaves to the sink tissues (e.g., roots, ears) is essential for maintaining plant growth and metabolism. However, the genetic basis underlying the establishment of sucrose transport capacity is poorly understood. As part of an effort to elucidate the genetic basis of this process, we have identified *carbohydrate partitioning defective* (*cpd*) mutants impaired in carbon allocation. *Cpd1* is a semi-dominant mutant that exhibits chlorotic leaf regions with excessive starch accumulation in the heterozygous state and seedling lethality in the homozygous state. Examination of the heterozygous individuals by light microscopy showed the presence of occlusions in the sieve elements of the phloem. Aniline blue staining and transmission electron microscopy determined that the occlusive material is callose. To examine the capacity for long-distance sucrose transport in the mutants, radiolabelling studies were performed and confirmed the impairment of sucrose movement out of the leaf. To further characterize *Cpd1* mutants, an RNAseq study was performed to identify transcriptional differences between *Cpd1* and wild-type leaves. Interestingly, this analysis revealed the up-regulation of genes involved in plant defense. Additional experiments showed an increase in reactive oxygen species in *Cpd1* leaves, consistent with a role for this gene in plant defense. To identify the *cpd1* gene, we have been conducting a map-based cloning strategy and are currently examining potential candidates. The current findings suggest that *cpd1* functions in both plant defense and the establishment of carbon flux within the plant, and the results indicate that impairment of carbon flux may function as a mechanism to prevent pathogen attack.

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P79

Changes in thiamine content in germinating maize lines: the effect of photoperiodic changes

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Maize is an important food crop not only because of its nutritive value (provides more carbohydrates than wheat and sorghum) but also a good source of thiamine. Maize and cornmeal constitute a staple food in many regions of the world. Thiamine (Vitamin B1) is a water-soluble vitamin and one of the important micro-nutrients required by living organisms for normal cellular function, growth and development as it plays a major role as a co-enzyme in carbohydrate metabolic pathways. Hence, it is important for the germination of seeds. The effect of photoperiodic changes on thiamine accumulation in three maize lines which were grown under ambient light and dark conditions was investigated. The results obtained confirms that thiamine is utilized during germination of maize and its utilization is significantly affected by photoperiodic changes which manifested as stunted and retarded growth in the germinating maize seedlings. Thiamine accumulation was more in maize seedlings under conditions of ambient light however best germination was achieved when grown under dark conditions.

Keywords: Maize, Thiamine, Photoperiodic, Germination, Accumulation.

P80

Characterization and mapping of *carbohydrate partitioning defective6*

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Through photosynthesis plants assimilate carbon dioxide into sucrose, which is subsequently transported, via the phloem, from the leaves of the plant to non-photosynthetic sink tissues for utilization in growth and development. When the plant is unable to transport sugars out of the leaf, starch accumulation occurs within the chloroplasts. Phenotypically, this excess in soluble sugars and starch can lead to leaf chlorosis, anthocyanin accumulation, and/or necrosis. We have identified multiple mutants manifesting these and other leaf phenotypes and have verified the occurrence of excess starch accumulation through potassium iodine staining. These mutants are collectively known as the *carbohydrate partitioning defective (cpd)* mutants. One such mutant displaying significant starch and anthocyanin accumulation in the leaf is *cpd6.* Bulk segregant analysis mapped the gene near the centromeric region of chromosome five. Fine mapping has since placed the gene within a region of about five centiMorgans. In this region there are approximately sixty-three genes, including ones involved in sucrose metabolism. In order to identify and clone the gene responsible for these phenotypes, we will continue to use molecular mapping methods to further fine map its genomic location and sequence candidate genes. Interestingly, in addition to excess carbohydrate accumulation in mutant leaves, defects have also been observed in the *cpd6* mutant vascular tissue. Identification of this gene should help elucidate the genetic pathways controlling the long-distance sucrose flux throughout the plant.

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P81

Characterization of drought stress response in two elite maize lines

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Drought is one of the major constraints of plant productivity worldwide. Understanding genetic and molecular mechanisms underlying the diverse strategies of drought tolerance is required in breeding for drought tolerance and genetic engineering of drought tolerant plants particularly with regard to climate change. We are characterizing two elite maize lines under drought conditions in the greenhouse. One line is of the dent pool and is highly adapted to drought prone areas in South Eastern Europe while the other one is of the flint pool and is not drought tolerant. For the dent line (RD) a decrease in stomatal conductance was observed at an earlier time point than for the flint line (DF). Similarly, the photosynthetic rate decreases earlier in RD than in DF and consequently the vegetative biomass of RD is more reduced under drought compared to well watered conditions than the biomass of DF. Quantification of ABA content in leaves showed that at six days after withholding water RD accumulated significantly more ABA than DF. We re-sequenced the genome of the two lines and are currently analyzing known drought stress associated genes for polymorphisms between RD and DF. Additionally, we aim to identify components of drought response mechanisms by using high throughput sequencing of mRNAs, long non-coding RNAs and small RNAs. Latest results on expression analyses of candidate genes will be presented.

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P82

Characterization of Maize Eukaryotic Translation Initiation Factor 5A Reveals Association with an Actin-Rich Cytoskeletal Fraction

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The eukaryotic translation initiation factor 5A (eIF5A) is a highly conserved protein among eukaryotes and is the only protein known to contain the unusual amino acid hypusine, which is synthesized as a post-translational modification of a conserved lysine residue and is necessary for the activation of eIF5A. It was first identified as a factor to promote the formation of the first peptide bond in rabbit. However, its functions in translation and cellular proliferation are poorly understood in plants. Maize eIF5A was observed to be one of several proteins that cause a mobility shift of labeled RNA derived from the 3' UTR of the maize 27 kDa gamma-zein gene. Further characterization shows that there are three genes coding for eIF5A in the maize genome and all three isoforms are expressed in tested maize B73 tissues, with eIF5A-1 and eIF5A-2 expressed at a similar level and eIF5A-3 expressed ten-fold lower. Western blot analysis shows increased protein accumulation in later stages of endosperm development, during which cells begin to undergo programmed cell death (PCD) in the central endosperm, suggesting a possible correlation of eIF5A and PCD in maize. Pulldown and immunoprecipitation analysis shows that eIF5A is associated with an actin and eEF1A rich protein fraction in maize endosperm. The association of eIF5A with this actin-rich fraction is hypusine independent. In contrast, the hypusine modification is required for association with ribosomes, consistent with prior experiments in yeast. Direct interaction of eIF5A with actin, eEF1A and eIF2A is confirmed by pulldown experiments.

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P83

Characterization of maize genes encoding plastidial ADPglucose pyrophosphorylase

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ADPglucose pyrophosphorylase (AGPase) is a rate-limiting enzyme for starch biosynthesis. Most AGPase is cytosolic in cereal endosperm, in contrast to the strictly plastidial location of this enzyme in other plant species and tissues. The function of the split localization of AGPase activity in cereal endosperm is unknown. This study addressed the starch biosynthetic functions of six maize genes encoding AGPase large- (AGPase LS) or small subunits (AGPase SS). Endosperm contained seven transcripts produced from these six genes, five of which could code for the remnant AGPase activity present when the cytosolic major form provided by *shrunken2* and *brittle2* is eliminated. The proteins encoded by all seven transcripts were expressed in *E. coli* and shown to be functional for AGPase activity when combined with partner subunits. Expression of GFP fusion proteins in maize protoplasts identified five AGPase subunits that are transported into plastids and confirmed the cytosolic locations of SH2 and BT2a. Null mutations were identified in the genes *embryoS* (*embS*) and *leafL*. The AGPase SS encoded by *embS* was necessary for accumulation of the majority of embryo starch, and the AGPase LS encoded by *leafL* was required for normal leaf starch content. Remnant starch was observed in both mutant tissues, indicating that additional genes also encode AGPase in embryo and leaf. Approximately 6% of the total starch in endosperm was conditioned by mutation of either *embS* or *leafL*, demonstrating that plastidial AGPase functions in seed starch production. Free amino acid levels in endosperm were altered by mutation of *embS*, including a 2- to 3-fold increase in glutamine content. Plastidial AGPase in endosperm is proposed to function in regulation of carbon flux between starch and other metabolites.

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P84

Characterization of Mutants Affecting Shoot Apical Meristem Function

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The shoot apical meristem (SAM) performs several functions that are critical for proper plant development. The network of genes that regulate the functions of the SAM are complex and not well understood. We discovered two new mutations in maize that affect two different functions of the SAM. One mutation is named *early flowering1* (*efl1*) and it affects the timing of the transition of the SAM from vegetative to reproductive phase. The lifetime of the plant is delicately balanced between vegetative and reproductive phases. In the case of *efl1*, mutant plants have an attenuated vegetative phase and transition faster to reproductive development. We will present results of our morphological and molecular analyses of this novel mutation. The other mutation is named *MATE leaf initiation* (*mli*) and it affects the rate of leaf initiation by the SAM. Morphological analysis of *mli* mutants demonstrates architectural consequences in changing the rate of leaf initiation. *mli* encodes a member of the MATE (multidrug and toxic compound extrusion transporter) protein family which is a large family of transporters of which their functions are mostly unknown. To the best of our knowledge, *mli* is the first MATE protein studied in maize that has been shown to have specific function in plant development.

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P85

Characterization of the Classic Maize Mutant *Albescent1*

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Albescent1 (al1) mutant seedlings tend to be green and become white over development due to photobleaching. The mutants can be rescued by growing under low light intensity to limit oxidative stress. The leaves of al1 plants accumulate phytoene, a carotenoid precursor; however, this is not due to a change in expression of *phytoene desaturase (PDS)*. The mutants also exhibit white endosperm, a trait originally ascribed to *y3*, which is now considered allelic to *al1*, and the two phenotypes are caused by pleiotropic effects. The locus was mapped to the short arm of chromosome two; in that region are two genes (GRMZM2G102349 and GRMZM2G010555) that show ~60% homology to photobleaching mutants characterized in Arabidopsis and tomato. These mutants have been determined to affect a plastid alternative oxidase (AOX) that accepts electrons from the plastoquinone pool to reduce oxidative damage under high light intensity. They may also be the alternative oxidase involved in chlororespiration. The putative maize *AOX* genes appear to be tandem duplicates, located less than 100kb apart on chromosome 2. The genes show 69% homology overall, and exons 5-8 are highly conserved. One maize paralog is more highly expressed than the other transcript, and both increase in abundance across developmental time, consistent with the increasing severity of the mutant phenotype over time. Seven mutant alleles have been discovered and maintained by the stock center. Additionally, six UniformMu insertion lines putatively contain inserts in the two gene models. We PCR amplified and Sanger sequenced the two regions to determine if there are causal changes in the coding regions of these two closely related genes. Structural changes in both genes may be able to determine if *al1* and *y3* are in fact distinct, but tightly linked genes.

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P86

Characterization of the Genes Responsible for *C*-Glycosyl Flavone Formation in Maize

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Maysin is a *C*-glycosyl flavone (CGF) shown to confer natural resistance against corn earworm (Helicoverpa zea) (CEW), a maize pest responsible for high yield losses per season. Maysin accumulates in silks, where it exerts its biocidal action by reducing CEW growth and development; and to a lesser extent in pericarps, the outermost layer of the maize kernel.

This research aims to determine the identity and function of the gene products involved in the last steps of maysin biosynthesis. Previous studies on QTL analysis have determined that the loci corresponding to the R2R3-MYB transcription factor P1 (or its duplicate P2) and the loci *salmon silks 1* (*SM1*), *salmon silks 2* (*SM2*) and *recessive enhancer of maysin 1* (*REM1*) are involved in the formation and accumulation of this CGF in silks. In addition, these studies also suggested possible functions for *SM2* being a rhamnosyl transferase, and *SM1* being involved in the last dehydration step towards maysin. However, there has been no activity proposed for *REM1*, nor where it is placed in the pathway and what might the identity of the genes involved in these conversions be.

Our search for possible candidate genes had the following criteria: They had to be included in the mapping intervals for those loci, their putative functions had to be similar to those proposed to *SM2* and *SM1* and they had to be highly expressed in P1-rr silks and pericarps. This candidate gene analysis yielded strong candidates for *SM2* and *SM1*, which are being biochemically characterized. This project is funded in part by NSF IOS-1125620.

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P87

Characterizing the expression pattern of *Sucrose Transporter1* and *Sucrose Transporter4* genes in maize

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To sustain the metabolism and growth of non-photosynthetic tissues, plants fix carbon in their leaves and transport it to distant tissues, primarily in the form of sucrose. In maize, sucrose is loaded into the phloem, moved through the veins, and unloaded into the sink tissues (e.g., roots, tassels, and ears) by sucrose transporters (SUTs). SUTs are a class of transmembrane-localized proteins that transport sucrose across a membrane using a proton gradient. In our lab, we have identified seven *Sut* loci in maize and are characterizing their function through both molecular and reverse genetic approaches. The *Sut1* gene is expressed in mature leaves and has been proposed to play a role in phloem loading. Meanwhile, *Sut4* is expressed in most tissues throughout the plant but has an unknown function. To further our understanding of the function of these *Sut* genes, a fluorescent reporter protein was translationally fused to the C-terminus of the SUT1 protein or placed under control of the *Sut1* or *Sut4* promoter regions and stably transformed into maize. In the case of *Sut1*, two successful transformations have been recovered: a full-length genomic SUT1-YFP (gSUT1-YFP) and a promoter Sut1-RFP (pSUT1-RFP). Meanwhile, one successful transformant has been found for *Sut4*: promoter Sut4-RFP (pSUT4-RFP). Fluorescent microscopic characterization of the cellular and subcellular expression patterns of these genomic and promoter fusion constructs will be presented. These studies will further our understanding of where the genes are expressed and function to transport sucrose within the plant.

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P88

Chloroplast-localized 6-phosphogluconate dehydrogenase is critical for maize endosperm starch accumulation

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Plants have duplicate versions of the oxidative pentose phosphate pathway (oxPPP) enzymes with a subset localized to the chloroplast. The chloroplast oxPPP provides NADPH and pentose sugars for multiple metabolic pathways. We identified two loss-of-function alleles of the *Zea mays* (maize) chloroplast-localized oxPPP enzyme, 6-phosphogluconate dehydrogenase (6PGDH). These mutations cause a *rough endosperm* (*rgh*) seed phenotype that reduces embryo oil and endosperm starch. Genetic translocation experiments show *pgd3* has separate, essential roles in both endosperm and embryo development. Endosperm metabolite profiling experiments indicate *pgd3* shifts redox-related metabolites and increases reducing sugars similar to starch biosynthetic mutants. Heavy isotope labeling experiments indicate carbon flux into starch is altered in *pgd3* with mutants having an increase of direct incorporation of glucose into starch. Labeling experiments with a loss of cytosolic 6PGDH did not affect flux into starch. These results support the known role for plastid-localized oxPPP in oil synthesis and argue amyloplast-localized oxPPP reactions are integral to endosperm starch accumulation in maize kernels.

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P89

Cloning of *Shattering1* suggests parallel selection during sorghum, rice, and maize domestication

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The loss of seed shattering is one of the key domestication syndrome traits that differentiate domesticated crops from their wild progenitors. Here, we show that seed shattering in sorghum is controlled by a single gene, *Shattering1* (*Sh1*), which encodes a YABBY transcription factor. Domesticated sorghums harbor three different mutations at the *Sh1* locus. Variants at regulatory sites in the promoter and intronic regions lead to a low level of expression, a 2.2-kb deletion causes a truncated transcript that lacks exons 2 and 3, and a GT-to-GG splice-site variant in the intron 4 results in removal of the exon 4. The distributions of these non-shattering haplotypes among sorghum landraces suggest three independent origins. The function of the rice ortholog (*OsSh1*) was subsequently validated with a shattering-resistant mutant, and two maize orthologs (*ZmSh1-1* and *ZmSh1-5.1+ZmSh1-5.2*) were verified with a large mapping population. Our results indicate that *Sh1* genes for seed shattering were under parallel selection during sorghum, rice and maize domestication.

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P90

Composition of nuclear mitochondrial DNA insertions on the short arm of chromosome 1 in B73

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The purpose of this study was to examine the composition of nuclear mitochondrial DNA insertion sites (NUMTs) on the short arm of chromosome 1 in B73. Previously, fluorescence *in situ* hybridization (FISH) identified a NUMT on chromosome 1S that was visible using the maize NB mitochondrial genome as a hybridization probe (Lough et al. 2008). This NUMT was not detected when shorter subsections of the mitochondrial genome were used as hybridization probes. Since FISH hybridization is not robust for probes smaller than 3 kb, we hypothesized that the B73 chromosome 1S NUMT is composed of many small pieces of mtDNA which could be rearranged and/or dispersed with other non-mitochondrial sequences. FISH studies demonstrate that the 1S NUMT is located distally to a subtelomeric repeat. Since the subtelomeric repeat is readily identified on maize genome assembly version B73 RefGen_v2, we used it as a proximal boundary in search of the 1S NUMT. Within this region of the genome assembly, we assessed the presence and organization of NB mitochondrial sequences using the ZeAlign BLAST program and the MaizeGDB Genome Browser. NUMTs were identified that included mitochondrial repeats, whole and fragmented mitochondrial genes, and chloroplast DNA. The largest contiguous mitochondrial DNA sequence was ~3.6 kb, and a total of ~29 kb from the NB mitochondrial genome was found dispersed in a region of the nuclear genome that is ~138 kb long. Multiple predicted genes and portions of retrotransposons and other repetitive DNA were also found in this region. Thus, the chromosome 1S NUMT is made of many small pieces of mtDNA dispersed within other nuclear sequences.

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P91

Conservation of ZmMYB31 and ZmMYB42 in the regulation of the maize lignin biosynthetic pathway among grass species

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Two R2R3-MYB type transcription factors (TFs) ZmMYB31 and ZmMYB42 are involved in the negative regulation of the lignin biosynthetic pathway. These TFs are 92% identical and we have shown that have both common and distinct target genes. SELEX assays were used to define the consensus DNA-binding site of ZmMYB31 and showed that it corresponds to the canonical AC-II element (ACCT/AACC) recognized by R2R3-MYB factors. Mobility shift assays indicated that both proteins strongly interact with the AC-II element of the maize COMT gene promoter in vitro, and that ZmMYB42 can also bind to an ACIII element. Chromatin immunoprecipitation (ChIP)-PCR and transient expression assays demonstrated that they share a set of common target genes in vivo. Both directly repress and interact with the lignin ZmCOMT and flavonoid ZmA1 genes promoters in vivo. Further results show that both proteins also bind to different targets, ZmMYB31 interacts with the ZmF5H gene promoter while ZmMYB42 interacts with the maize Zm4CL2 gene promoter in vivo. The combined results show that these closely related TFs play non-redundant functions in the complex regulation of the phenylpropanoid pathway in maize. We have now generated antisera against MYB31 and MYB42 homologs from sorghum, rice and switchgrass and are investigating if this regulation is conserved amongst different grass species. This project is currently supported by NSF grant IOS-1125620 and previously by DBI-0701405.

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P92

crw1- A Novel Maize Mutant Highly Susceptible to Foliar Damage by the Western Corn Rootworm Beetle

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Western corn rootworm (WCR), *Diabrotica virgifera virgifera* Leconte (Coleoptera: Chrysomelidae), is the most destructive insect pest of corn (*Zea mays* L) in the United States. The adult WCR beetles (WCR beetles) derive their nourishment from multiple sources including corn pollen and silks as well as the pollen of alternate hosts. Conversely, the corn foliage is largely neglected as a food source by WCR beetles, leading to a perception of a benign interaction between the two. We describe here a novel recessive mutation of corn that was identified and named after its foliar susceptibility to corn rootworm beetles (*crw1*). The *crw1* mutant under field conditions was exceptionally susceptible to foliar damage by WCR beetles in an age-specific manner. It exhibits pleiotropic defects on cell wall biochemistry and morphology of leaf epidermal cells and lower structural integrity via differential accumulation of cell wall bound phenolic acids. These findings indicate that *crw1* is perturbed in a pathway that was not previously ascribed to WCR susceptibility, as well as implying the presence of an active mechanism(s) deterring WCR beetles from devouring corn foliage.

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P93

Development of metabolomic methods for the simultaneous identification of polar and non-polar surface lipid metabolites: A gateway to the further understanding of cuticular lipid biosynthesis in maize silks

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During the critical period of pollen reception, the cuticle of maize silks protects against abiotic and biotic stresses such as UV radiation, desiccation, pathogen invasion, and insect feeding. The research goal of this multidisciplinary team is to identify and characterize the genetic and metabolic networks responsible for the production of surface lipids (SLs) on maize silks. Silk SLs comprise mostly unbranched, linear hydrocarbons (HCs), including 65 constituent metabolites that are 14 to 35 carbon alkanes, alkenes or dienes. The remaining SLs comprise fatty acid (FA) precursors and aldehyde/ketone intermediates of the hydrocarbon biosynthesis pathways, which remain enzymatically undefined. Although the biological production of linear hydrocarbons occurs via the conversion of saturated and unsaturated FAs, neither the biochemical mechanisms, nor the genetic elements involved in this biochemical transformation have been defined in plants. To distinguish among several potential reaction networks that underlie SL biosynthesis, our team leverages genetic variation across maize genotypes, as well as morphogenetic variation during silk development to determine the reaction steps, as well as the enzymes and regulators involved. Because these are large-scale efforts, we require metabolomic methods for simultaneously profiling the ~100 non-polar and polar SL metabolites we have observed. Here we present our progress in developing an extraction method that permits accurate quantification of both polar and non-polar SL metabolites. We have used a range of solvents, varied solvent incubation times, performed extractions with and without silica, and identified derivatizations necessary for efficient GC-MS quantification. For validation, we are applying these methods to experiments involving genetic and developmental variation in silk SL composition.

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P94

Defining the role of Prolamin-box binding factor-1 (pbf1) gene during maize domestication

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Maize was domesticated from the wild grass, teosinte (Zea mays ssp. parviglumis) ~6300 years ago. Many traits were under selection during domestication including the starch and protein content of the kernels. One method of identifying genes that contribute to trait differences between maize and teosinte is to look for the signature of past selection using nucleotide diversity data. The prolamin-box binding factor-1 (pbf1) gene encodes a transcription factor, which controls the expression of two classes of zein genes, the 27 kDa γ- and 22 kDa α-zein, in maize and teosinte. Two prior studies found evidence that pbf1 underwent selection during maize domestication. In order to determine if pbf1 controls a phenotypic difference between maize and teosinte, we assayed a maize-teosinte BC2S3 family segregating for a 6.33 MB teosinte segment (Chr 2: 151,570,555-157,901,437) including the teosinte pbf1 allele in an isogenic background. The progeny homozygous for the teosinte allele had a small but significantly higher kernel weight (162 mg ±1.17) than homozygous progeny with the maize allele (156 mg ± 1.07). Protein assays showed no difference in zein content between lines carrying the maize vs. teosinte allele of pbf1. RNA-seq was performed using RNA from 16 DAP kernels for isogenic lines with maize vs. teosinte allele and reciprocal F1s of these lines. The RNA-seq data failed to show any difference in zein gene regulation by the maize vs. teosinte allele of pbf1. Possible alternatives explanations for the difference in seed weight that we observed include: (1) PBF is regulating other endosperm-specific genes that control seed weight or (2) a gene in the introgressed segment other than pbf controls this phenotype.

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P95

Dehydration Stress Memory in Arabidopsis and Maize

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We recently observed Arabidopsis (Ding et al, 2012) and maize (unpublished) plants subjected to a first dehydration stress and a one day watered recovery period, had altered physiological and transcriptional responses during a second dehydration stress. Plants subjected to a previous dehydration/watered recovery cycle (termed 'trained' plants) maintained a higher turgor pressure in a subsequent dehydration stress than 'untrained' plants experiencing their first dehydration stress. This difference in turgor pressure during dehydration stress was due to differences in the rate of water loss. We consider this a form of physiological memory in the trained plants, as they fully recover their turgor pressure during the watered recovery period between the times the dehydration stresses are applied, and yet have a different physiological response in the next dehydration stress.

Dehydration stress memory is also associated with transcriptional changes (Ding et al, 2012). We have recently performed whole genome transcriptome analyses by high-throughput cDNA sequencing (RNASeq) for Arabidopsis and maize plants (unpublished data). RNA was isolated from plants subjected to the 3 following stages: non-stressed watered (W); a first dehydration stress (S1); or a second stress (S2) after a watered recovery interval. The W to S1 comparison elucidates the untrained dehydration stress response. The S1 to S2 comparison allows for identifying trained genes with altered responses during their second dehydration stress. These studies demonstrate that a large number of genes in both species show transcriptional memory as evidenced by their trained responses when comparing transcript levels in S1 vs S2. We see both positively (up regulated) and negatively (down regulated) training, as expected for such a large number of trainable genes. Updates on the mechanism involved will also be discussed.

Ding Y, Fromm M, Avramova Z (2012) Multiple exposures to drought 'train' transcriptional responses in Arabidopsis. Nature communications 3: 740

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P96

Deregulating Cysteine and Methionine Biosynthesis in Maize

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It has previously been shown that constitutive expression of bacterial sulfonucleotide reductases in maize chloroplasts resulted in significant increases in the rate of sulfur assimilation and cysteine/methionine accumulation in kernels, but with growth inhibition resulting from the accumulation of toxic intermediates. The evidence suggests that cell-specific gene expression and removal of metabolic intermediates through adjustment of other metabolic steps might alleviate growth inhibition and further enhance methionine accumulation. Towards this goal we examined the effect of expressing Pseudomonas aeruginosa 5'-adenylylsulfate reductase or Escherichia coli 3'-phospho-5'-adenylylsulfate reductase (EcPAPR) in combination with Arabidopsis serine acetyltransferase (AtSAT1) in either bundle sheath under control of the RbcS promoter, mesophyll cells under control of the PepC promoter, or general expression under control of the Ubi promoter. Transgenic lines of maize Hi-II B x A were isolated for all constructs with a range of expression levels of each transgene. In general, it was found that bundle sheath expression of PaAPR alone or in combination with AtSAT1 does not alleviate the toxic effect of deregulating sulfur assimilation. However, bundle sheath expression of EcPAPR and AtSAT1 results in the deregulation of sulfur assimilation with reduced impact on plant growth. By generating plants that overexpress EcPAPR or PaAPR in a tissue-specific or constitutive manner, we are also trying to determine which of these combinations is able to decouple increased sulfur assimilation from phenotypic aberrations. Currently, cell-specific expression of EcPAPR in the mesophyll or bundle sheath cells shows plant phenotypes that range from normal to severe (e.g., presence of white sectors). Analysis of protein expression levels and localization are in progress. Additional results concern the effects of transgenic expression of sulfur assimilation enzymes in mesophyll cells and general expression in all cell types.

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P97

Distinct functional properties of isoamylase-type starch debranching enzymes in monocots and dicots

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Isoamylase-type starch debranching enzymes (ISA) play important roles in starch biosynthesis as shown by strict conservation in chloroplast-containing species of both catalytically active ISA1 and the non-catalytic homolog ISA2. Functional distinctions exist between species, however, that are not understood. ISA1 and ISA2 in dicot leaf and tuber are each required at the same step for normal starch biosynthesis, presumably functioning in a heteromulitmeric complex. In contrast, maize and rice endosperm exhibit near-normal starch metabolism without ISA2. This study took *in vivo* and *in vitro* approaches to determine whether tissue-specific physiology or evolutionary divergence in ISA1 structure is responsible for distinctions in ISA function. ISA1 was shown to be enzymatically and physiologically active in maize leaves lacking ISA2, as in endosperm. Maize ISA1 was expressed in *Arabidopsis* lacking endogenous ISA1, or lacking both native ISA1 and ISA2. Unlike *Arabidopsis* ISA1, the maize protein functioned in *Arabidopsis* leaves to support normal starch accumulation in the absence of ISA2. Recombinant *Arabidopsis* ISA1 required ISA2 as a partner for enzymatic function whereas recombinant maize ISA1 was active alone. These results support the conclusion that maize ISA1 has evolved to function without ISA2, yet ISA2 remains conserved in the Poaceae. Presence of ISA2 affected starch granule size and number in maize or *Arabidopsis* leaf and maize endosperm. Biophysical measurements indicated maize ISA1 is an elongated homodimer with distantly spaced active sites. By extension, inclusion of ISA2 together with ISA1 in a heteromultimeric complex could provide variable active site spacing, and thus contribute to modeling of precursor homoglucan architecture prior to crystallization and higher order assembly into granules. This may explain conservation of the non-catalytic ISA2 protein, by allowing flexibility in starch granule size to meet the requirements for glucose storage and release under varying physiological conditions.

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P98

Divergent Transcriptional Responses between Maize Genotypes Resistant and Susceptible to MDMV infection

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Maize resistance to viruses has been well-characterized at the genetic level, and loci responsible for resistance to potyviruses including *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV), have been mapped in several genotypes. For instance, *Mdm1*, conferring potyvirus resistance in Pa405, has been mapped to the short arm of chromosome 6, with genes on chromosomes 3 and 10 also contributing. However the mechanisms of virus resistance in maize are not well understood. Map-based cloning approaches have been attempted to isolate the gene(s) responsible for conferring resistance to SCMV, but have not yet been fully successful. Moreover, how resistance genes trigger downstream mechanisms to confer resistance are not well understood. In this study, we used Illumina next generation sequencing to examine global transcriptional responses between maize genotypes that are resistant (Pa405 and Oh1VI) and susceptible (Oh28 and B73) to MDMV infection. Our results identify candidate genes and cellular pathways that may contribute to MDMV resistance and should be the subject of downstream approaches.

Funding acknowledgement: United States Department of Agriculture (USDA)

P99

DROPS: An EU-funded project to improve drought tolerance in maize

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DROPS (DROught-tolerant yielding PlantS; www.drops-project.eu) is an EU-funded project that will develop novel methods and strategies to improve maize performance under water-limited conditions. An interdisciplinary approach based on high-throughput phenotyping under controlled and field conditions will generate data that will be used for ecophysiological modeling to predict the performance of maize and wheat under fluctuating water regimes. The identification of Quantitative Trait Loci (QTL) for morpho-physiologcal traits that influence yield under drought conditions will produce additional data for modeling maize performance based on QTL effects. The project targets root architecture, transpiration efficiency, vegetative growth maintenance and seed abortion. In particular, DROPS will:

- Develop new screens that will consider indicators which are (i) highly heritable and measurable in a high-throughput fashion in phenotyping platforms; (ii) based on metabolite concentration, sensitivity parameters of models or hormonal balance; (iii) genetically related to target traits and able to predict genotype performance in the field via simulation and/or statistical models;

- Explore the natural variation of the target traits by (i) linking the target traits to physiological pathways, genes or genomic regions (ii) assessing the effects of a large allelic diversity for the four target traits via association genetics;

- Support crop improvement strategies by developing methods for estimating the comparative advantages of relevant alleles and traits in fields with contrasting drought scenarios. This will be achieved via field experiments and by developing new crop models able to estimate the effects of alleles on maize growth, yield and water-use efficiency.

Funding acknowledgement: The DROPS project is funded by the European Community's Seventh Framework Programme under the grant agreement n° FP7-244374.

P100

Fall Armyworm induced *Mir1* (Maize Insect Resistance 1) gene expression in Maize Inbred Lines

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Nearly 100 insect pests are known to infest maize (*Zea mays*), one of the most important crops worldwide. The annual crop loss to animal pests in maize is 16%, majority of which is due to insects. Maize plants are known to put up an arsenal of defense responses in response to herbivory by insects such as caterpillars, aphids, rootworms, etc. A resistant inbred line Mp708, developed from the Antiguan population by classical plant breeding, is known to accumulate a 33kD cysteine protease Mir1-CP, which disrupts the peritrophic matrix of fall armyworm caterpillars (*Spodoptera frugiperda*) and severely retards their growth. Mp708 plants with detached roots, showed significantly lower Mir1 accumulation in the whorls suggesting possible movement of the protein from the roots to leaves upon infestation by fall armyworm. Resistance to fall armyworm caterpillars was evaluated in a wide array of maize inbred lines developed in southern USA (Mp708, Mp496, Tx601, AB24E), mid-western USA(B73, Mo17, Oh43) and CIMMYT in Mexico (CML67, CML139, Ki3) by feeding whorl tissue to caterpillars. *Mir1* transcript accumulation was also measured in these lines in both roots and leaves after 24 hours in response to aboveground fall armyworm herbivory in the whorls. Inbreds resistant to FAW herbivory such as Mp708, Mp496 showed *mir1* transcript accumulation in shoots and roots in response to aboveground herbivory while most of the susceptible lines did not. AB24E, a susceptible inbred known to accumulate an inactive 36kD Mir1 protein, showed *mir1* transcript accumulation. Interestingly, another susceptible inbred Oh43, showed significantly higher *mir1* transcript levels in both tissues in uninfested plants compared to ones infested with fall armyworm. This suggests the possible role of caterpillar secretions (such as saliva or from the ventral eversible gland) or excretion in successfully suppressing plant defenses.

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P101

Farnesyl diphosphate synthase 3 (FPPS3) is responsible for the production of herbivore-induced terpene defenses

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Volatile terpenes play an important role in the chemical defense of maize plants against a variety of biotic and abiotic stresses. Maize plants attacked by caterpillars release a mixture of mono- and sesquiterpenes which attract parasitic wasps that are specific 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.

The first step in the biosynthesis of volatile sesquiterpenes, the formation of farnesyl diphosphate (FPP), is catalyzed by prenyl transferases. In the maize genome, we found one previously characterized prenyl transferase (Li and Larkins, 1996; Cervantes-Cervantes *et al.* 2000) and two closely related genes. Heterologous expression in *E. coli* and subsequent biochemical characterization of the enzymes revealed that FPPS1 and FPPS3 produce FPP. The analysis of transcript levels indicated that the expression of these two *fpps* genes is induced by herbivory, although *fpps1* and *fpps2* are only marginally expressed. To find the FPP synthase responsible for sesquiterpene production *in planta*, we searched for quantitative trait loci (QTL) of volatile production that correspond to the FPPS locations. In a Nested Association Mapping (NAM) population screened for herbivore-induced volatile production, we identified a QTL closely related to FPPS3 that corresponded to the production of the major sesquiterpenes (*E*)-nerolidol, (*E*)-ß-farnesene and (*E*)-α-bergamotene. Within the NAM population, KY21 has a low emission of sesquiterpenes and contributes to the significance of this QTL. However, KY21 expresses the same allele of FPPS3 at a similar level but shows reduced expression of other terpene biosynthesis genes. This suggests that the QTL corresponds to a regulatory element that does not affect FPPS3 activity directly but rather other factors that regulate herbivore defense.

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P102

Fine Mapping *carbohydrate partitioning defective7*

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Carbon partitioning, the process in which sugars are moved from photosynthetic source tissues to non-photosynthetic sink tissues (e.g., developing leaves, ears), is essential for plant growth and development. Despite its importance, the details of carbon partitioning, as well as the genes involved in the process, are not fully understood. A *carbon partitioning defective* (*cpd*) mutant, *cpd7*, has been identified by its yellow source leaves, which turn red due to anthocyanin accumulation. Starch staining these leaves also revealed that they hyperaccumulate starch. From its leaf coloration and starch accumulation phenotypes, we have determined that *cpd7* is involved with carbon partitioning. To identify the causative mutation, we are applying a positional cloning strategy. Using Bulk Segregant Analysis (BSA) mapping, *cpd7* has been mapped to chromosome 9. Through PCR-based mapping techniques, we have narrowed the genomic location to approximately 2.3cM. Ongoing efforts aim to find the gene responsible for the mutation. Independent mutant alleles will be characterized for verification that the correct gene has been identified. This information will help determine which genes function in whole-plant carbohydrate partitioning.

Funding acknowledgement: National Science Foundation (NSF)

P103

Flooding Responses in Maize: Molecular Characterization and Genetic Variation

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Maize is highly susceptible to flooding, especially during early vegetative development. We reasoned that knowledge of flooding response strategies from rice (*Oryza sativa*) and Arabidopsis (*Arabidopsis thaliana*) as well as maize genetic diversity may be used to improve flood tolerance in maize. In rice, the group VII ethylene responsive transcription factor (G7ERF) SUBMERGENCE1A (SUB1A) confers a quiescence submergence response strategy [Xu et al. 2006; Fukao et al., 2006]. In Arabidopsis, G7ERFs regulate survival of oxygen deprivation, a stress endured during flooding [Gibbs et al., 2011; Licausi et al., 2011]. Examination of the B73 maize reference genome identified 14 G7ERFs of which three *SUB1A-like ERFs* (*SUB1-like ERF [SBL]1-3*), along with the low-oxygen marker gene *Alcohol dehydrogenase1* (*Adh1*) increased mRNA in shoot tissue following 1-3d submergence at the four-leaf (v4) stage. Arabidopsis G7ERFs are unstable in well-aerated tissue, as they are substrates of the N-end rule pathway of targeted proteolysis. The N-terminal cysteine of these proteins is necessary for their oxygen-mediated turnover [Gibbs et al., 2011; Licausi et al., 2011]. Oxygen-mediated turnover of G7ERFs can be recapitulated in a rabbit reticulocyte *in vitro* transcription translation assay. Using this approach, we confirmed that at least five maize G7ERFs are substrates of N-end rule degradation. Several of these are highly induced by flooding at the seedling and v4 stage of development. Identification of the B73 submergence response both at the transcriptomic and translatomic level is underway, along with evaluation of NAM lines displaying extremes in survival of submergence during germination and at the v4 stage. This research was supported by the US National Science Foundation grant MCB-1021969 and Pre-doctoral Fellowship DGE-0813967.

Funding acknowledgement: National Science Foundation (NSF)

P104

From phenotype to genotype by high-throughput genetic analyses of Mutator transposons

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Large populations of tagged mutants and methods for mining them have spurred major advances in functional genomics of model organisms. Transposon tagging in particular has been invaluable for maize, especially with the advent of high-throughput sequencing methods for mapping of insertions in high-copy transposon systems. Here we adapt Mu-Seq technology, developed for use with the UniformMu public resource, for fast and efficient genetic analysis of phenotype-genotype relationships. We use Mu-Seq to dissect Mu-insertion profiles of 12 families selected for visible kernel phenotypes from the UniformMu population. Using Mu-Seq profiling, 282 segregating Mu insertions were simultaneously genotyped in 141 individual plants, and tested for co-segregation with kernel phenotypes. Co-segregation was observed for seven genes, including at least five that share putative roles in organeller mRNA processing. Phenotype-to-genotype genetic analyses are thus enabled for simultaneous appraisal of many individuals and families carrying high-copy transposon systems in maize, and potentially other species.

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

P105

Functional Genomics of Sugar Content in Sweet Sorghum

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Sweet sorghum [Sorghum bicolor (L.) Moench] represent an ideal crop for the production of bio-ethanol. It has a sugar-rich stalk and produces high biomass with low input. In addition, it is widely adaptable, grows rapidly, requires minimal fertilization, and because it is a drought tolerant C4 crop, it has high water-use efficiency and can be planted in non-arable and marginal lands. Moreover, it has a diploid genome that has been sequenced making it a good model system for the more complex genomes such as the polyploid sugarcane. Compared to grain sorghum, sweet sorghum is taller, has a smaller panicle, produces lower grain yield, and more importantly, it accumulates significantly higher sugar concentrations in the stem, which suggests that there are differences in carbon flux between the two lines. Thus, using both grain and sweet sorghum to conduct this study will lead to a better understanding of the regulation of carbon partitioning within plants. Sucrose transporters (SUTs) function to transport sucrose across plant cellular membranes. Six SUTs have been identified in the sorghum genome, but limited data is available on the function of these genes. This research project is aimed at understanding the mechanisms that regulate carbon allocation to the stem of sweet sorghum. Our specific goals are to: 1) characterize the expression and function of SUTs in different sorghum tissues and thus determine which are involved in sucrose accumulation in the stem, carbon export in the leaves, as well as accumulation in other sink tissues, such as roots and seeds; and 2) measure bioenergy-relevant traits such as plant height, dry matter, and gas exchange related parameters, that could be critical in understanding the accumulation of carbohydrates in sorghum. Results from this research could lead to the improvement of sucrose accumulation in the stem of sweet sorghum.

Funding acknowledgement: Department of Energy (DOE)

P106

GA biosynthetic deficiency is responsible for maize dominant Dwarf11 (D11) mutant symptom: Physiological and transcriptomic evidence

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Dwarf stature is introduced to improve lodging resistance and harvest index in crop production. In many crops including maize, mining and application of "new" dwarf genes is urgent to overcome genetic bottleneck and vulnerability during crop improvement and breeding. We report the characterization and expression profiling analysis of a newly identified maize dominant Dwarf11 (D11) mutant. The D11 displayed severely developmental abnormalities, including shortened internodes, white leaf margins, multiple degenerated spikes, etc. Evidence from segregation ratios in backcross and self-pollination populations, in combination with the phenomenon that the mutant phenotype was firstly observed in F1 progeny, demonstrated that the D11 mutant symptom is controlled by a dominant Mendelian factor. The D11 mutant in response to both GA3 and paclobutrazol (PAC) stimulation indicated that dwarfism symptom of the D11 mutant is attributed to GA biosynthesis instead of GA signaling pathways deficiency. In contrast, maize dominant dwarf plants D8 and D9 are all insensitive to exogenous GA3 application. Additionally, allelic test showed that D11 is not allelic to D8 and D9. Microarray and qRT-PCR results demonstrated that transcripts encoding GA biosynthetic and catabolic enzymes ent-kaurenoic acid oxidase (KAO), GA 20-oxidase (GA20ox), and GA 2-oxidase (GA2ox) were up-regulated in the D11 mutant. Results presented here lay a foundation for the following D11 cloning and functional characterization.

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P107

Generation of allelic diversity in *ZmPtf1* to produce variation in maize response to phosphorous starvation.

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ZmPtf1 encodes a bHLH transcription factor, identified by homology to a previously characterized rice gene that is involved in tolerance to Pi starvation. Overexpression of *Ptf1* in both maize and rice results in significantly higher root length, root surface area, and higher Pi uptake rate under low phosphate conditions. Furthermore to play an important role in the regulation of carbon partitioning. It is difficult, however, to determine gene function from overexpression alone. Nor does overexpression allow us to assess the contribution of diversity in *Ptf1* to standing variation in Pi starvation tolerance or the potential utility of such variation in crop improvement.

To investigate further the role of *ZmPtf1* in tolerance to Pi starvation, we have iniciated a program of insertional mutagenesis *ZmPtf1* using the *Ac/Ds* transposon system.

ZmPtf1 is locate on Chromosome 9S within the interval defined by the kernel markers Shrunken *(Sh)* and Bronze *(Bz)*.By taking advantage of well characterized *Ac* and *Ds* insertions in the *Bz* locus, I have used a genetic strategy to obtain efficiently novel insertional mutants through selection of local transposition events. By selecting either revertant or stable *bz* exceptions from the *bz-m2::Ac* mutable allele, I am generating enriched my screening populations prior to PCR based screening *ZmPtf1*. In addition, I have selected a number of events for mapping using a test cross to a line carrying the *bz-m2(DI) Ds* derivative. On the basis of mapping results, I will further enrich my populations for PCR-screening, and select events for direct cloning of *Ac* insertions, allowing me to recover insertions outside of the immediate gene space covered by my screening primers.

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P108

Genetic diversity assessment among yellow endosperm tropical-adapted maize inbred lines using SSR and allele specific PCR-based markers

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Maize is a major cereal crop in many parts of sub-Saharan Africa. Vitamin A deficiency is prevalent among many pre-school children and women of reproductive age in sub-Saharan Africa. The consumption of yellow endosperm maize cultivars containing high level of β-carotene can reduce vitamin A deficiency. A total of 122 tropical-adapted yellow endosperm maize inbred lines were genotyped with 62 SSR markers to assess the genetic diversity and allele specific PCR-based markers of the carotenoid candidate genes, lycopene epsilon cyclase (LCYE) and β-carotene hydroxylase 1 (crtRB1) were validated among the inbred lines. In total, 51 SSR loci were polymorphic and 190 alleles were detected with an average of 3.72 alleles per locus and PIC values among inbred lines varied from 0.12 to 0.74 with an average of 0.43. Genetic distance (GD) values among all pairs of 122 inbred lines varied from 0.02 to 0.61 with an average of 0.41 for the SSR markers. The maize inbred lines exhibited a substantial level of genetic diversity, cluster and principal coordinate analyses based on SSR GD estimates revealed clear separation of tropical-adapted maize inbred lines into three groupings consistent with pedigrees of the inbred lines. The three groups formed based on SSR have the maize inbred lines with best allelic variants for LCYE 5'TE and 3'TE polymorphisms while those containing best alleles for the Indel4, 3'TE, 5'TE polymorphisms in the crtRB1 gene were found only in group III of the SSR grouping. The SSR and gene-based data can be used for accurate selection of diverse parental lines.

Keywords: Genetic diversity . SSR markers . Tropical-adapted maize inbred lines . Pro-vitamin A . Allele-specific PCR-based markers

P109

Genetic diversity assessment among yellow endosperm tropical-adapted maize inbred lines using SSR and allele specific PCR-based markers

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ABSTRACT- Maize is a major cereal crop in many parts of sub-Saharan Africa. Vitamin A deficiency is prevalent among many pre-school children and women of reproductive age in sub-Saharan Africa. The consumption of yellow endosperm maize cultivars containing high level of β-carotene can reduce vitamin A deficiency. A total of 122 tropical-adapted yellow endosperm maize inbred lines were genotyped with 62 SSR markers to assess the genetic diversity and allele specific PCR-based markers of the carotenoid candidate genes, lycopene epsilon cyclase (LCYE) and β-carotene hydroxylase 1 (crtRB1) were validated among the inbred lines. In total, 51 SSR loci were polymorphic and 190 alleles were detected with an average of 3.72 alleles per locus and PIC values among inbred lines varied from 0.12 to 0.74 with an average of 0.43. Genetic distance (GD) values among all pairs of 122 inbred lines varied from 0.02 to 0.61 with an average of 0.41 for the SSR markers. The maize inbred lines exhibited a substantial level of genetic diversity, cluster and principal coordinate analyses based on SSR GD estimates revealed clear separation of tropical-adapted maize inbred lines into three groupings consistent with pedigrees of the inbred lines. The three groups formed based on SSR have the maize inbred lines with best allelic variants for LCYE 5'TE and 3'TE polymorphisms while those containing best alleles for the Indel4, 3'TE, 5'TE polymorphisms in the crtRB1 gene were found only in group III of the SSR grouping. The SSR and gene-based data can be used for accurate selection of diverse parental lines.

Keywords: Genetic diversity . SSR markers . Tropical-adapted maize inbred lines . Pro-vitamin A . Allele-specific PCR-based markers

P110

Genetic Investigation of Temperature Sensitivity of a Maize Autoimmune R-gene

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The autoactive Rp1-D21 allele in maize triggers a spontaneous hypersensitive response, or HR, in which necrotic lesions, typical of those produced in the event of pathogen invasion, begin forming even in the absence of pathogen. HR in plants is equivalent to the innate immune response in animals and it involves quick collapse of one or few plant cells at the site of infection by a wide variety of pathogens. It has been demonstrated in past research that the phenotypic severity of Rp1-D21 lesions are affected by factors such as genetic background and temperature at which the plants are grown, some being greatly suppressed and others being enhanced to the point where plant death results.
In this study, Rp1-Kr1N, a new allele of Rp1 that also confers autoimmune HR, was accessed for its sensitivity to temperature. The goal of the research was to determine over what temperature range HR is suppressed and where it is enhanced in certain genetic backgrounds. Hybrid plants possessing the Rp1-Kr1N allele in a some select genetic backgrounds of the NAM founder inbreds were grown in a controlled environment of a growth chamber and closely observed for HR lesions at different temperatures. Plants were scored on a scale that qualitatively represented the severity so accurate comparisons could be made between plants. Results reveal that elevated temperatures above 20°C suppress HR while temperatures of 20°C below enhance the phenotype. It is believed that the data gathered from this experiment on HR phenotypic expression in relation to temperature will help elucidate the effect of environmental factors on HR and other genes in the future.

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P111

ZmSBP30 is closely associated with the number of rows of kernels and strongly selected during domestication and improvement of Zea mays

(submitted by Yonglian Zheng)

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Five single nucleotide polymorphisms (SNPs) significantly associated with kernel row number (KRN) on chromosome 4 were identified using a genome-wide association study. The SNPs formed a linkage disequilibrium (LD) block, which was placed in a consensus QTL interval identified earlier. The stronger-than-expected shared synteny suggests that syntenic genes in the LD block have evolved in parallel across five grass species and also that zmSBP30, an ortholog of OsSPL14, is an important candidate gene for regulating the development of kernel rows, which can be thought of as branches of the maize ear. Furthermore, the gene association study also showed a close association between zmSBP30 and KRN, and revealed the causal sites and allelic effects which were supported by linkage mapping using four populations representing combinations of different alleles. A marker-aided backcrossing program confirmed that Haplotype 1 of ZmSBP30 markedly increased KRN. Nucleotide diversity showed that zmSBP30 has been strongly selected during domestication and improvement of maize, and Haplotype 1 allele has enriched the temperate lines of maize.
Keywords: maize (Zea mays L.), kernel row number, SBP-box protein, single nucleotide polymorphism (SNP), genome-wide association study, parallel evolution, inflorescence

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P112

Genetic variability analysis of candidate domestication loci in 5,100 BP maize samples from San Marcos cave, Tehuacán.

(submitted by Miguel Vallebueno Estrada <mvallebueno@langebio.cinvestav.mx>)

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Humans have contributed to the domestication of many animal and plant species around the planet since the early Holocene, with important consequences for the processes that shape genomic structure in relatively fast evolutionary context. The origins of maize can be traced back to a single domestication event originating from populations of *Zea mays ssp. parviglumis* of the Balsas region around 9000 years ago. Although the significant reduction in nucleotide variability that affects ~4% of maize genome could be associated with a domestication bottleneck (Yamasaki *et al* 2007), a clear understanding of genetic function, ancient allelic variability, and time for selection remains to be elucidated for most of the corresponding loci. Under the guidance of the Instituto Nacional de Antropología e Historia (INAH), we initiated the exploration of previously discovered or unreported rock shelters potentially containing paleobotanic plant samples in central and southeast Mexico. The first season in Tehuacan valley yielded more than 30 non-manipulated maize samples that were morphologically characterized and carbon dated using accelerator mass spectrometry (AMS). Their age spans several thousand years, with specific strata yielding equivalent age at distinct geographic locations. Using paleogenomic approaches, DNA was extracted from the oldest samples found in San Marcos cave (5,000 - 5,100 BP), with total yields allowing multiple library construction for next-generation sequencing technologies, without maize DNA enrichment. Their initial alignment to sequences coalescing around previously identified domestication loci suggests recovery of informative segments corresponding to ancient allelic variants. We anticipate that their analysis will allow comparisons of genetic variability between ancient maize samples and extant landraces, allowing a glimpse of the impact exerted by ancient maize populations in current genetic diversity.

Funding acknowledgement: Consejo Nacional de Ciencia Y Tecnología (CONACYT)

P113

Genome-Wide Association Study for Tocopherols Content and Compositions in Maize Kernel

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As known as vitamin E, tocopherols play an important role in maintaining human's health. Compared with other staple crops, maize contains a higher level of tocopherols including γ-tocopherol and α-tocopherol in its kernels. The rich variation of tocohperols content and composition with high heritability contributes to understand the genetic architecture of tocohperols in maize kernels, since the relevant metabolic pathway has been elucidated in Arabidopsisand Synechocystis. In this study, a genome-wide association study (GWAS) was conducted with a panel of 513 diverse maize inbred lines. Two sets of genotype were used in this study, namely 56110 SNPs from Illumina MaizeSNP50 Beadchip and 0.55 million high-quality SNPs derived from RNA-sequencing with a MAF of 0.05. In this study, we manage to attain objectives as follows: 1) to identify genetic loci affecting tocopherols content and composition; 2) to dissect the genetic architecture of tocopherols in maize kernels; 3) to develop useful markers aiming at high vitamin E maize breeding. In total, 29 independent significant loci were identified through GWAS, including 7 for α-tocopherol, 8 for γ-tocopherol, 11 for total tocohperol content and 7 for the ratio of α/γ-tocopherol. Hopefully the accumulation of favorable alleles may help increase the tocopherols content and achieve a healthier ratio of different forms of tocopherols. Furthermore, this study may provide a simple guideline for breeders to improve the content and composition of tocopherols in maize.

Funding acknowledgement: National Science Foundation (NSF)

P114

Genomic Distribution of Maize Facultative Heterochromatin Marked by Trimethylation of H3K27

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Trimethylation of histone H3 lysine 27 (H3K27me3) plays a critical role in regulating gene expression during plant and animal development. We characterized the genome-wide distribution of H3K27me3 in five developmentally distinct tissues in maize plants of two genetic backgrounds, B73 and Mo17, representatives of two distinct heterotic groups. There are numerous differences in the distribution of H3K27me3 among tissues. In contrast, we found the distribution of H3K27me3 among the two genetic backgrounds to be quite similar. The tissue-specific patterns of H3K27me3 are often associated with differences in gene expression among the tissues and most of the imprinted genes that are expressed solely from the paternal allele in endosperm are targets of H3K27me3. Many maize genes with important developmental roles, including numerous genes encoding putative transcription factors, are modified with H3K27me3 in at least one of the tissues that were profiled. A comparison of the H3K27me3 targets in rice, maize, and Arabidopsis provided evidence for conservation of the H3K27me3 targets among plant species. However, there was limited evidence for conserved targeting of H3K27me3 in the two maize subgenomes derived from whole genome duplication. Genomic profiling of H3K27me3 in loss-of-function mutant stocks for *Mez2* and *Mez3*, two of the three putative H3K27me3 methyltranferases present in the maize genome, suggests partial redundancy of this gene family for maintaining H3K27me3 patterns. Only a portion of the targets of H3K27me3 requires *Mez2* and/or *Mez3* and there was limited evidence for functional consequences of H3K27me3 at these targets. This study provides a catalogue of 6,337 genes that are marked by H3K27me3 in five maize tissues. 4r

Funding acknowledgement: National Science Foundation (NSF)

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P116

Got Starch? Decoding the *Carbon partitioning defective4 (Cpd4)* mutant gene in maize

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The process by which carbon is transported from source tissues (photosynthetic leaves and tissues exporting carbon) to sink tissues (carbon-importing tissues) has been characterized at the physiological and biochemical levels; however, the genes responsible for this process have yet to be identified. Knowledge of the genes controlling this pathway could allow the development of new approaches for genetically modifying crops, which could lead to increased crop yield and improved human health. Optimizing the potential for crop production has benefits in both agriculture (food and cattle management) and biofuel production. To identify these genes, maize plants are first screened in the field for phenotypic indications of carbon accumulation in leaves (chlorosis, anthocyanin accumulation, decreased plant height, etc.) and are then starch stained for confirmation as carbon partitioning defective (Cpd) mutants. *Cpd4* is a mutation phenotypically identified in the field which conditions excess starch accumulation in yellow leaf tissues. To understand the function of the *cpd4* gene, we are cloning it using a map-based approach. Genomic DNA is isolated from *Cpd4* mutants and wild-type individuals, and then analyzed by PCR with polymorphic DNA markers and gel electrophoresis in order to identify recombinant individuals. In turn, these recombinant individuals are then used to delineate the chromosomal region where the *cpd4* gene is located. By examining the DNA sequence in this interval, candidate genes will be identified and sequenced to determine which one is responsible for the *Cpd4* mutant phenotype. Once the *cpd4* gene is identified, it can be manipulated with the goal of increasing carbon partitioning in the plant and therefore increasing sucrose and starch concentrations in the sink tissues for increased yield and biomass.

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P117

Hunting the recessive *Carbon partitioning defective5* mutant in maize

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Carbon partitioning is the process by which fixed carbon (e.g. sugars) is transported from photosynthetic source tissues (mature leaves) to non-photosynthetic sink tissues (e.g. developing leaves, ears). Understanding this process is of fundamental importance not only because of its vital role in plant growth and development, but also because of the human dependence on plants for food and energy. However, although carbon partitioning has been extensively studied, little is known about the regulatory genes that control it. Through previous large-population screenings, we have identified maize mutants that display a carbohydrate accumulation phenotype (i.e., an excess buildup of starch and soluble sugars is observed in the source leaf tissues of these mutants). In an effort to further understand this carbon accumulation we identified various recessive mutations which prevented the export of carbon from the source tissues. One of these carbon partitioning defective (cpd) mutations, *cpd5*, was identified as a mutant that displayed yellow source leaves, which later turned red as a result of anthocyanin accumulation. We mapped this cpd mutation to its general location within the maize genome, to enable the map-based cloning of the gene, and found that the *cpd5* mutation mapped to chromosome 4. Using known DNA markers, we are continuing to map the mutation to a specific region. As a result of this mapping, we plan to sequence and eventually clone the gene underlying the *cpd5* mutation. These findings will provide valuable biological insights into how plants control the distribution of fixed carbon, and may enable us to manipulate the maize genome for greater crop yield in an effort to feed and fuel the world's growing population.

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P118

Identification of a genetic modifier that interacts with *bm4* to regulate plant height

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The brown midrib mutants (*bm1-bm6*) are important models for the study of lignin biosynthesis. After backcrossing reference alleles of most of the brown midrib mutants into the B73 inbred background, segregation for stunted plants was observed in F_2 families segregating for *bm4*, but not other *bm* mutants. In these F_2 families only *bm4* mutant plants exhibited the stunted phenotype. Our segregation and genetic mapping data are consistent with the existence of a B73-derived modifier that interacts with the *bm4-ref* allele to reduce plant height. RNA samples from midribs of separate pools of stunted and non-stunted *bm4* mutant F_2 plants derived from the cross *bm4-ref*/*bm4-ref* x B73 were subjected to BSR-Seq (Liu et al., 2012) analysis. BSR-Seq, a type of bulked segregation analysis (BSA), makes use of RNA-Seq reads to identify SNPs and subsequently map genes responsible for mutant phenotypes. As expected based on the known location of the *bm4* locus, both pools were enriched for non-B73 derived SNPs on the long arm of chromosome 9. In contrast, the stunted pool preferentially inherited B73-derived SNPs along the remainder of chromosome 9; whereas, the non-stunted pool preferentially inherited non-B73 derived SNPs along the entire chromosome. This result suggests that a B73-derived modifier is located on chromosome 9. Previously, Vermerris et al. (2010) reported that *bm2*; *bm4* double mutants exhibit a stunted phenotype. Because *bm2* is located on chromosome 1, the modifier reported in the current study is unlikely to be related to *bm2*. Future plans for this project include fine mapping and eventual cloning of the modifier, which may elucidate novel interactions affecting lignin biosynthesis.

P119

Identification of differentially expressed genes in root tissues of sorghum lines known to have high or low nitrogen use efficiency

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Nitrogen (N) is one of the most important nutrients limiting crop growth and yield but crop plants utilize less than 30% of N applied. A vast amount of excess N is lost through leaching and contributes to environmental pollution. There is a critical need to develop the genotypes with improved nitrogen use efficiency (NUE) for input efficient, environmentally friendly sustainable agriculture. Here, we attempted to find differentially expressed candidate genes using Illumina RNA-seq by comparing root tissues of sorghum genotypes, Ck60 (low NUE), BTx623 (low NUE reference), San Chi San, China17 (high NUE china lines), KS78. We also compared bulks of five high-NUE and five low-NUE RILs (Ck60 x San Chi San) selected based on yield performance under limited N conditions. Pair-wise comparisons of low-NUE versus high-NUE genotypes and bulks of RILs led to the identification of 183 genes whose expression was changed at least four-fold in at least six of the 12 high NUE versus low NUE pairwise comparisons. 38 common differentially expressed genes were found in at least nine pairwise comparisons. We are using qRT-PCR to validate selected differentially expressed genes. Some of these genes could provide insight into the molecular basis of NUE and suggest ways for improving NUE in sweet sorghum as an efficient bioenergy crop.

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P120

Identification of expression regulatory hotspots in developing maize kernel

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Maize kernel is of agronomic importance due to its role in maize grain yield and maize nutrition. Numerous genes contribute to the regulatory network of the formation of kernel shape and the accumulation of maize components. Here, we performed genome-wide association studies (GWAS) to detect expression quantitative trait loci (eQTL) using 1.03 million single nucleotide polymorphisms (SNPs) and the expression profiles of 28,769 genes gained from RNA Sequencing of maize kernel at 15 days after pollination in 368 maize inbred lines. In total, we identified 349 regulatory hotspots which can simultaneously regulate the expression of more than or equal to ten genes at $P<8.93\times10^{-8}$. Among the top 12 eQTL, *eq6* was regulated by *eq4*, *eq4* as well as *eq9-2* were regulated by *eq7-1*, and *eq7-1* was regulated by itself. It seems that *eq7-1* is likely a central locus in expression regulation. *Eq1-1* and *eq9-1* mutually regulated each other, and a group of genes were simultaneously regulated by these two loci. It indicates these two loci may play their biological role in a synergetic way. Besides, *eq3-2*, *eq5*, and *eq7-2* could regulate downstream genes through regulating the expression of themselves. To further investigate the potential traits that these regulatory hotpots my affect on, the correlation between the expression of co-regulated genes and a series of maize kernel related traits were carried out. We found the genes regulated by *eq7-2* were highly negatively correlated with one hundred kernel weight (HKW) and kernel width (KW), and positively correlated with oil concentration and embryo to endosperm ratio (EER). The genes regulated by *eq4* are negatively correlated with kernel length (KL). Maize mutants will be used to validate the important role of these regulators in expression regulation and the specific traits they affect on.

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P121

Identification of presence absence variation in the landrace Palomero Toluqueño

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Recent increase in the availability of genomic data has revealed the high occurrence of presence absence variation (PAV; a particular sequence present in some individuals and missing in others) in maize, and it has been hypothesized that PAV may be functionally significant, possibly playing a role in adaptation to specific environments. In our work, we are studying PAV in the Palomero Toluqueño (PT) maize landrace with respect to modern improved inbreds. The aims are 1) to demonstrate the existence of PAV in PT relative to the B73 reference genome, 2) to investigate the distribution of PAV sequences within PT accessions and more broadly within the genus *Zea* and 3) to establish tools to map and link PAVs to phenotypic differences in PT. To identify candidate PAV sequences from PT, a subset of contigs has been selected from the transcriptome of a B73 x PT F1 hybrid individual on the basis of good alignments to PT whole genome sequences, and poor-alignment to the B73 reference genome and further maize genomic data-sets. From this a subset of 12 sequences have been confirmed as high confidence PAVs on the basis of genomic PCR analysis. The distribution of these high-confidence PAVs is now being characterized in further PT accessions, other landraces and teosintes, demonstrating presence absence variation of these 12 sequences among landraces, teosintes and inbreed lines. B73 x PT mapping populations are also being developed to allow genetic mapping of PAV loci and to investigate linkage to phenotypic traits of agricultural and evolutionary importance.

P122

Identification of QTLs for herbivore-induced terpene production by Nested Association Mapping (NAM) and Genome Wide Association Studies (GWAS)

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Terpenes are secondary metabolites essential for many plant defense strategies. When attacked by herbivores, maize plants emit a mixture of volatile terpenes. They attract natural enemies of the herbivore, thus reducing caterpillar damage to the maize plant. The volatile terpenes are produced by terpene synthases which are strongly induced after herbivory. Our aim is to identify the signal transduction pathways that control the emission of herbivore-induced volatiles.

To identify components of herbivore-induced terpene production, Nested Association Mapping was performed with about 5000 recombinant inbred lines derived from 26 parent lines. The lines were screened for herbivore-induced volatile emission in order to identify Quantitative Trait Loci (QTLs). One QTL corresponded to the production of several mono- and sesquiterpene volatiles, Nerolidol, DMNT, Linalool, Farnesene and Bergamotene. Genome-Wide Association Studies (GWAS) was used for close mapping of QTL 215 and identified several SNP-markers within 1 MB distance to the QTL. Since Nerolidol, Linalool, Farnesene, and Bergamotene are produced by separate pathways and enzymes, the QTL correlates most likely with a regulatory element of terpene biosynthesis. Often, the transcript levels of regulatory genes are induced by the same cue. In the region of the QTL, two herbivore regulated genes were identified; one gene coding for a kinase, the other one coding for a pentatricopeptide. These candidate genes will be investigated for their role in the regulation of terpene biosynthesis.

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P123

Introgression of o2 allele in endosperm of normal maize using molecular marker approach

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Maize endosperm comprises of four proteins namely prolamines, glutelins, albumins and globulins. Predominant protein 'prolamins' constituting 50-60% of the total protein content of maize endosperm, is low in two essential amino acids lysine and tryptophan. Consequently, the biological value of maize based food remains to be relatively poor. The version of maize called quality protein maize (QPM), which involves opaque2 (o2) allele and other endosperm modifiers is rich in lysine and tryptophan. The o2 encodes a transcription factor which increases the content of non zein proteins particularly, EF-1 alpha, which is positively correlated with lysine content in the endosperm and reduces 22-kD alpha zeins. QPM has usable protein as energy in range 8.3-9.6% well above the estimate of protein and energy need of a one year old child. Malnutrition accounts for nearly 50% of the child deaths in India, more than 1.5 million children are at the risk of becoming malnourished because of rising global food prizes. Our present investigation thus aims at taking a step forward in solving this problem by carrying out the conversion of normal maize into QPM using sequence repeat based SSR markers. Normal maize inbred lines having high per se performance were used in conversion programme as recurrent parent and CML lines, developed at CIMMYT, were used as donor parent for o2 allele. Polymorphism was detected between parental lines using gene specific markers, phi057 and umc1066. These markers were further used in foreground selection in BC1F1 and BC2F1 during the backcross programme and plants heterozygous for opaque2 gene were selected. Selected plants of BC2F1 population were self pollinated to generate BC2F2 seeds having o2 allele in homozygous condition. The BC2F2 seeds were screened using light box for characterization of individual kernel based on different degree of endosperm modification. Maize kernels from each plant were therefore grouped as less than 25%, 25–50% and more than 50% kernel modification. The kernel having 25% or less modified endosperm were selected for further evaluation of morphological and biochemical parameters.

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Investigating the role of the chloroplast chaperone BSD2

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Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the rate-limiting step in carbon fixation. It is widely desired to increase Rubisco activity or efficiency; however there has been little success due to poor understanding of the Rubisco assembly pathway. One Rubisco assembly factor is defined by the *Bundle Sheath Defective 2 (bsd2)* mutant, which specifically fails to accumulate Rubisco. BSD2 is a chloroplast protein with some similarity to DnaJ chaperones and was therefore proposed to act as a Rubisco assembly factor, or as a translational chaperone for the Rubisco large subunit in bundle sheath chloroplasts, where Rubisco is exclusively located. Curiously, however, both proteomic and immunoblot assays reveal that BSD2 is present at similar levels in mesophyll and bundle sheath chloroplasts. This suggests that BSD2 has a previously unappreciated function in mesophyll chloroplasts. We have taken a transgenic approach to elucidating its function by expressing BSD2 from cell-type specific promoters, and introducing these cassettes into the *bsd2* mutant background. Bundle sheath-expressed BSD2 is expected to complement Rubisco deficiency, but should fail to complement any role of BSD2 in the mesophyll. Possible mesophyll roles include repression of Rubisco LS accumulation, or folding of non-Rubisco substrates.

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P126

Investigation of the role of a Divaricata type transcription factor in Zea mays

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The first member of the DIVARICATA (DIV) sub-family of MYB transcription factors (TFs) was discovered in Antirrhinum majus and found to play a role in dorsoventral symmetry. The DIV TF sub-family is small but well conserved in plants and study of a DIV TF in tomato revealed that it appears to affect cell division and expansion. Little or no study of DIV TFs has been performed in monocots. This family of TFs exhibits two MYB DNA-binding domains of which the second contains a characteristic SHAQKY motif. We surveyed the repertoire of DIV TFs in maize and related grasses and found that there are at least five complete DIV genes none of which has been studied to date. We report here a phylogenetic comparison of this subfamily in monocots. In addition we availed of the Ac/Ds tagging project at Cornell and identified a Ds insertion in exon2 of one of these genes (ZmDIV6). The insertion is immediately adjacent to the SHAQKY motif and thus likely to knock out gene function. These plants were selfed and grown to identify a possible phenotype. Here we will report on our characterization of this mutant and our analysis of DIV6 gene expression in different plant tissues during maize development. We have subcloned the DNA binding portion of this protein and are attempting overexpression of the protein for DNA biding studies. This project was funded by in part by grant NSF DBI-0701405, IOS-1125620, and by the Ohio Plant Biotechnology Consortium.

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P127

Maize *Brittle1* and *Brittle2* genes polymorphisms bioinformatic analysis

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Brittle endosperm mutations in maize have significant effect on endosperm maturation, altering its structure. Indeed, *bt1* allele increases the concentration of adenosine 5[prime] diphospho-glucose (ADP-Glc) in 13 times compared to wild type mutants. *Brtittle2* mutants are known for reduced activity of ADP glucose pyrophosphorylase enzyme because of altered ADP glucose pyrophosphorylase structure. Phenotypically, Brittle mutations manifested in modified endosperm structure, which leads to its brittleness. Aim of our research is to determine natural *Brittle1* and *Brittle2* polymorphisms, as well as its homologs within *Poaceae* family. 113 *Brittle2* gene nucleotide sequences and 12 of *Brittle1* ones (from NCBI) were used for analysis. All nucleotide sequences, as well as translated ones were aligned using ClustalW via MEGA 5. UPGMA method was used to observe evolutionary history of *Brittle1* and its paralog – *Brittle2* gene, as well as their orthologs within *Poaceae* family. Multiple alignment data were used to build corresponding phylogenetic trees. Ideas about possible *Brittle1* and *Brittle2* evolution were proposed. Trees were concordant with known phylogenetic data, tribes *Panicoidae* and *Triticae* formed separate clusters. Tajima test for evolution neutrality was conducted. Negative Tajima D value indicated non-neutral character of *Brittle1* and *Brittle2* genes evolution, domestification process interference was proposed. Primers, specific to maize *Brittle1* and *Brittle2* genes polymorphic nucleotide sequences, as well as *Poaceae* – specific primers were designed using FastPCR program. Maize-specific primers were used to reveal interspecific *Brittle1* and *Brittle2* polymorphism, *Poaceae*-specific ones were used to reveal intraspecific polymorphism respectively. In silico PCR showed good segregating potential of designed primers, with aim of maize-specific primers we were able to differ maize lines by *Brittle1* and *Brittle2* allele spectrum. Subsequent in vitro PCR reaction, using designed primers would be able to differ maize genotypes with *Brittle1* and *Brittle2* allele spectra.

P128

Maize genes encoding the carotenoids biosynthesis enzymes polymorphisms

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The problem of quality and balanced diet is extremely important for Ukraine. Its decision is possible by realization of biofortification strategy to reduce the specific deficiencies in the diet, especially iron, zinc and vitamin A. One of the most consumable cereal is maize. However, despite the wide variety of carotenoids in the grain, including a precursor of vitamin A, maize can not fulfill the needs of this substance, becouse of the carotenid that giving the highest yield of vitamin A - beta-carotene is converted to the less efficient carotenoids, such as beta-cryptoxanthin and zeaxanthin, by biochemical changes in plants. That is why, actualities are genetic and bioinformatics studies of genes encoding of maize carotenoid biosynthesis enzymes. Our research aim is to analyze the polymorphism of seven genes encoding key enzymes of carotenoid biosynthesis in maize grain: psy1, vp5, y9, zds, vp7, lcye and hyd3. By polymerase chain reaction method DNA typing of 28 maize lines and hybrids, that differ in endosperm color (white-yellow-orange) performed for different regions of the genes (promoters, exons, introns, microsatellite loci). The polymorphism was marked for most studied genes in the maize genotypes. Identifying sets of amplicons, which are polymorphic among the analyzed samples and related to the level of carotenoids in the grain, will let devise system of molecular markers for screening of initial breeding material and selection of forms with high content of beta-carotene.

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P129

Maize genes involved in carbon partitioning

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Many of the genes involved in moving photoassimilate from source to sink and in initiating carbon reallocation during development remain unknown. As part of a larger project to understand how these processes are controlled genetically, we have screened EMS mutagenized populations for mutants that show characteristic, carbon partitioning defective phenotypes in the leaf and tassel. Over 200 of these mutants have been used to generate F2 mapping populations, at various stages of analysis. In addition, crosses of the two carbon-partitioning mutants *tie-dyed1* and *tie-dyed2* to the NAM founders have identified phenotypic variation indicating genetic modifiers. Finally, we have mapped a mutation in the inbred NC300, which shows a tie-dyed like phenotype in older leaves that appears to become suppressed in the upper plant. This mutation maps to the same region of chromosome 6L as *tdy1*; interestingly, this mutation does not appear to be allelic to *tdy1*.

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P130

Maize nested-association-mapping (NAM) founder lines exhibit diverse responses to caterpillar feeding

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Maize (*Zea mays ssp. mays*), one of the most important crops worldwide, has to face attacks from various insects during its life cycle in the field. The major pests of maize include caterpillars, beetles, aphids, and thrips. There is little study concerning the natural resistance mechanism of maize to these herbivores. Traditional plant breeding has developed a series of maize inbred lines (Mp496, Mp704 and Mp708) resistant to fall armyworm (FAW) and southwestern corn borer (SWCB). A 33-KD cysteine protease, Mir1-CP, was discovered to be a major player in the inhibition of caterpillar larva growth. Caterpillar-resistant maize lines are able to transcribe *mir1* mRNA and generate Mir1-CP under caterpillar attack, while unfortunately caterpillar-susceptible maize lines appear to have lost this ability.

Modern maize was domesticated from teosinte and *Zea mays ssp. parviglumis* was the direct ancestor of maize. During this process, maize has maintained high level of genetic diversity. Maize nested-association-mapping (NAM) founder lines were selected to capture and represent such enormous genetic diversity. However, there is little research about how this genetic diversity contributes to maize insect resistance traits.

Insect bioassays have shown that NAM founder lines exhibit different levels of constitutive resistance to FAW. RNA sequencing analysis of *mir1* transcription has shown that it is only expressed in the root and tassel tissue of few NAM founder lines (MaizeGDB). Insect bioassay also showed that *Zea mays ssp. parviglumis* and *Zea mays ssp. mexicana* have resistance levels that are lower than those of Mp708.

In the future, we would like to study the defense response of teosinte to herbivory and what type of selection *mir1* has experienced during domestication from teosinte to modern maize.

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P131

Manipulation of *candyleaf1* affects biofuel quality of maize cell walls

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A maize mutant, *candyleaf1 (cal1-R),* was identified in an EMS screen of A619, due to its increased mixed-linked glucan (MLG) content and increased digestibility after saccharification. Interestingly, in *cal1* mutants, glucan content is highest early in development but increased levels are maintained after senescence and the plants have no visible phenotype, difference in kernel yield or biomass. Thus, after ears are harvested for food or feed, stover could be harvested as biofuel. A second allele verified that *candyleaf1* encodes a mixed-linked glucan endoglucanase. Expression analysis revealed this second allele to have reduced transcript and F1s generated in complementation tests had similar cell wall composition as *cal1-R* homozygotes. To explore the function of the *cal1* enzyme, overexpression constructs were transformed into maize using *Agrobacterium.* In wildtype, there is a spike of MLG in cell walls of second leaves. Consistent with our hypothesis that constitutive high expression of *cal1* would preclude the MLG spike by degrading glucans immediately, T1 plants had reduced levels of glucose compared to wildtype sibs containing no transgene. T2 plants are growing and are expected to complement the *cal1-R* mutation. Potential uses of *cal1* to improve maize stover as a biofuel will be discussed.

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P132

Maximizing the Reliability of Genomic Selection by Optimizing the Calibration Set of Reference Individuals: Comparison of Methods in Two Diverse Groups of Maize Inbreds (*Zea mays* L.)

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Genomic selection refers to the use of genotypic information for predicting breeding values of selection candidates. A prediction formula is calibrated with the genotypes and phenotypes of reference individuals constituting the calibration set. The size and the composition of this set are essential parameters affecting the prediction reliabilities. The objective of this study was to maximize reliabilities by optimizing the calibration set. Different criteria based on the diversity or on the prediction error variance (PEV) derived from the realized additive relationship matrix–best linear unbiased predictions model (RA–BLUP) were used to select the reference individuals. For the latter, we considered the mean of the PEV of the contrasts between each selection candidate and the mean of the population (PEVmean) and the mean of the expected reliabilities of the same contrasts (CDmean). These criteria were tested with phenotypic data collected on two diversity panels of maize (*Zea mays* L.) genotyped with a 50k SNPs array. In the two panels, samples chosen based on CDmean gave higher reliabilities than random samples for various calibration set sizes. CDmean also appeared superior to PEVmean, which can be explained by the fact that it takes into account the reduction of variance due to the relatedness between individuals. Selected samples were close to optimality for a wide range of trait heritabilities, which suggests that the strategy presented here can efficiently sample subsets in panels of inbred lines. A script to optimize reference samples based on CDmean is available on request.

P133

Modular Recombination Cloning as a Method for High-Throughput Vector Construction

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To meet the growing need for increasing number of transgene vectors , we have developed a modular and high-throughput cloning process using recombination cloning methods (RCM). Traditional cloning methods allow only one DNA fragment to be inserted into a plasmid per cloning step and requires multiple cloning sites within the plant-transcriptional unit (PTU). The RCM permits simultaneous cloning of multiple fragments that are recombined using short DNA sequence homologies present between adjacent fragment ends. Additionally, RCM avoids extraneous DNA sequences, such as multiple cloning sites, from vectors and unnecessary modification of genes and regulatory elements to eliminate restriction enzyme sites. We have created a modular cloning process by adding standard end homology sequences for each recombination fragment that can easily be added through PCR amplification. With this system we are capable of designing a high-throughput automated vector assembly system that is compatible with robotics.

P134

Molecular characterization of maize Rp1-D21-regulated hypersensitive response

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The maize Rp1-D21 gene confers a hypersensitive response (HR) in the absence of pathogen infection. Rp1-D21 came from a recombination event between Rp1-D, a NB-LRR protein, which confers rust resistance to corn, and Rp1-dp2, a homologue of Rp1-D. We have previously shown that H2O2 accumulation, the increased expression of PR1 and other defense marker genes (PRms and WIP1) were associated with Rp1-D21-mediated HR phenotype. We also demonstrated that the phenotype is both temperature- and genetic background-dependent. Here we demonstrate a strong association between the strength of the Rp1-D21-associated HR in different genetic backgrounds and salicylic acid accumulation, defense gene expression. In addition, we demonstrate that the Rp1-D21-regulated HR is light-dependent. To investigate the genetic loci modulating Rp1-D21-regulated HR, we analyzed the differential expressed genes (DEGs) between WT and mutant from the whole transcriptome level by RNA-seq. Preliminary analysis showed that the DEGs involved diverse pathways. Transient expression of Rp1-D21 in N. benthamiana induced clear HR. A serial of deletion constructs from different domains of Rp1-D21 did not show HR, which indicated that the HR phenotype required the full-length of Rp1-D21. Domain swaps between Rp1-D and Rp1-dp2 indicated that the NB and the N-terminus of LRR domains from Rp1-dp2 are important for Rp1-D21-mediated HR. Rp1-D21 was found to be mainly localized in cytoplasm, and the cytoplasm distribution was required for the HR phenotype. We will further elucidate the transcriptional network and biochemical characterization of Rp1-D21-associated HR.

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P135

Molecular Characterization of Maize Sucrose Transporters, *ZmSut2* and *ZmSut4*

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Carbon assimilation and sucrose transport are key to sustaining plant life. Although we know a great deal about the initial biochemical reactions from carbon dioxide assimilation through sucrose synthesis, little is known about the control of long-distance sucrose transport in plants. In maize (*Zea mays* L.), seven *sucrose transporter* (*Sut*) genes have been identified through genomic comparisons to known *Suts* of other species. To date, only *ZmSut1* has been extensively studied. Unlike its orthologs in sugarcane and rice, *Sut1* was shown to function in phloem loading of sucrose in maize leaves. To better understand the functional role of each of the remaining maize *Suts*, and because orthology does not necessarily indicate function, we set out to identify *Mutator* (*Mu*) transposable element insertions in the other six *Sut* genes to characterize the consequences of the loss of their functions. We have identified two independent *Mu* insertions in both *ZmSut2* and *ZmSut4*, which are proposed to be null mutations, due to the insertions mapping within predicted protein coding regions. Using RT-PCR and qRT-PCR techniques, we are characterizing the molecular expression patterns of the *Sut* genes in both mutants and wild-type plants. Our expression studies support that these insertions are null mutations; however, the mutants have no visibly obvious plant growth defects. To investigate the possibility of genetic compensation among the *Sut* genes, qRT-PCR was performed on the *sut* mutants and revealed functional genetic redundancy among some of the *Sut* genes. These and other experiments will help determine the biological functions of the maize *Suts*.

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P136

Molecular interactions of RTCS and RTCL: conserved and specific features of two paralogous LOB domain proteins in maize (*Zea mays* L.)

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Maize has a complex root system made up by the embryonic primary root and seminal roots, and an extensive postembryonic, shoot-borne root system. Shoot-borne crown and brace-roots make up the major backbone of the root system. *Rtcs (Rootless concerning crown and seminal roots)* encodes a LBD (Lateral Organ Boundaries Domain) protein that regulates shoot-borne root initiation in maize. RTCS and RTCL are closely related paralogs that share an overall protein identity of 72% and 88% identity of their LOB domains. Molecular interactions and functional features of these two paralogous LBD proteins were compared in this study. Overall *Rtcs* displays on average a significantly higher transcript abundance than *Rtcl* in the analyzed root tissues of 3 to 8-day-old maize seedlings. *Rtcs* is highly expressed in coleoptilar nodes in which crown roots are initiated whereas *Rtcl* is not. Both RTCS and RTCL show preferential binding to DNA fragments containing *LBD* motifs. Three protein-protein interactors of RTCS or RTCL that were identified via yeast-two-hybrid screening were validated via bimolecular fluorescence complementation (BiFC) experiments in protoplasts, including a metallothionein like protein (MEL), a Glycine rich RNA-binding protein (GLYC) and a stress responsive protein (STR). The protein interactions are dependent on the C-terminal regions of LBD proteins and can be affected by the subcellular localization of the interacting proteins. Moreover, the expression of *Rtcs* and *Rtcl* is auxin inducible. Luciferase reporter assays showed that ARF34 (AUXIN RESPONSE FACTOR 34) can bind to the promoters of both genes and activates their expression. In summary, the presented data characterized novel conserved and specific features of RTCS and RTCL, two paralogous LOB domain proteins in maize.

P137

Mutational analysis of pyruvate orthophosphate dikinase (PPDK) function in maize leaves and endosperm

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Pyruvate orthophosphate dikinase (PPDK) catalyzes the reversible phosphorylation of pyruvate and orthophosphate (P_i) by ATP to form phosphoenolpyruvate (PEP), pyrophosphate (PP_i), and AMP. This reaction is integral to C4 metabolism, but PPDK is also present at high levels in endosperm where its metabolic function is not known. This study isolated transposon insertion mutations of *pdk1* and *pdk2*, the two maize genes that encode PPDK, and also reduced or eliminated PPDK expression in endosperm by tissue-specific expression of RNAi molecules that targets transcripts of both genes. The mutation *pdk1:MuEx10* caused seedling lethality, whereas *pdk2:DsEx4* did not affect viability or noticeably influence plant growth. Transgenic plants expressing the RNAi construct from the 27 kDa zein promoter lacked detectable PPDK protein in endosperm, yet did not affect plant growth or viability. RNAi expression from the 22 kDa zein promoter caused strongly diminished PPDK expression compared to sibling kernels lacking the transgene, although the proteins were reproducibly detected. Complete loss of PPDK in endosperm caused significant changes in free amino acid abundance, including a major increase in alanine. Similar but quantitatively diminished effects were observed in endosperm with reduced PPDK expression. These data indicate elevated pyruvate concentrations because alanine is produced in single step from pyruvate. Increased alanine abundance in the absence of PPDK, therefore, indicates that the direction of the reaction in endosperm normally is from pyruvate to PEP, which is inconsistent with a potential glycolytic function of the enzyme. These data provide direct evidence that PPDK function in endosperm affects amino acid metabolism. PPDK is located in both the cytosol and amyloplasts, so it could be an important determinant of PP_i concentration in the stroma and thus affect other metabolic fluxes in endosperm tissue. Starch content was not altered, however, in immature endosperm lacking PPDK.

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P138

Novel evidence of Al tolerance in maize supported by ZmNrat1 candidate gene

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Aluminum (Al) toxicity is a major factor limiting agricultural productivity in acid soils. In low pH soils, Al assumes phytotoxic effects causing a rapid inhibition of root growth that consequently decreases water and minerals uptake. Al exclusion mechanism based on Al-activated organic acid release from root apices has been the main mechanism of Al tolerance in plants. However, plants can neutralize internal Al once inside the cytosol. This physiological mechanism has recently been supported by the identification of other genes associated with Al tolerance, including Nramp aluminum transporter in rice (Nrat1). Nrat1 is an Al3+ transporter located in the plasma membrane of root apical cells, suggesting its putative role in the internal Al detoxification mechanism. In maize, the Al tolerance is complex, suggesting several genes associated. Indeed, ZmMATE1 is the only gene associated with Al tolerance in this species so far. Here, we present a putative maize homologue gene, sharing over 80% of amino acid sequence similarity to rice Nrat1, which was mapped in a genomic region highly associated with Al tolerance in a RIL population derived from a cross between Cateto Al237 (Al tolerant) and L53 (Al sensitive). This candidate gene, called ZmNrat1, was specifically expressed in roots, and differentially expressed along the first three centimeters of root tips after Al treatment. Furthermore, the Al tolerant line Cateto Al237 showed an early induction of ZmNrat1 in the presence of Al when compared to the Al sensitive line, L53. This expression pattern was highly consistent with the expression of the MZ00052211, a probe representing this candidate gene, in a microarray experiment using root tips of contrasting lines under toxic level of Al in nutrient solution. Finally, the expression pattern of ZmNrat1 was highly consistent with the rice Nrat1, suggesting its putative association with Al tolerance in maize.

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P139

Only 50% of maize ovaries give rise to fully developed seed.

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In studies of a transgenic *shrunken-2* gene that increases seed number in wheat, rice and maize, we (Hannah et al., 2012. Plant Cell 24:2352) recently found that only about one-half of maize ovaries give rise to a fully developed kernel. Here we asked (i) if this is also true in the two popular inbreds, B73 and Mo17 and (ii) if seed development/ non-development occurred at particular locations on the cob.

Similar to our earlier findings, only 45% (209/462) of the Mo17 ovaries and 52% (369/708) of the B73 ovaries gave rise to fully developed kernels. Most non-development occurred in the top and bottom quarters of the ear. Only 22.4% and 19.8% of ovaries in the upper quarter of Mo17 and B73 ears, respectively, produced fully developed kernels. Corresponding percentages for the bottom quarter were 41.4% and 44.8%. These patterns were seen as early as 8 days post-pollination.

As judged from pollinated and non-pollinated ears harvested at the time of maturity, the act of pollination or kernel development leads to a loss of detectable ovaries and poorly developed seeds. This was particularly prevalent in the base of the ear.

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P140

Phylogenomic analysis of the Trihelix transcription factor family in grasses.

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The Trihelix (THX) family of transcription factors (TFs) has been described only in land plants, and may therefore be involved in plant-specific processes. There is experimental evidence from Arabidopsis and rice that these roles are mainly in flower, fruit, and seed development. This family has not been well investigated and most THX proteins are of unknown regulatory function. THX TFs exhibit one or two trihelix DNA-binding motifs that bind to GT cis elements to regulate transcription. We have taken advantage of the near complete maize genome to identify at least 27 trihelix family members in corn and have performed a phylogenomic comparison to those in rice, sorghum, and Brachypodium. We used the sequence of full length cDNAs and the maize genome to confirm gene models for these THXs. We report on the conservation of this family across multiple monocot and dicot species. We also find that the THX motif is present in lower land plants such as Physcomitrella but not in any algal species suggesting that family arose to regulate land plant specific processes. This project is part of the GRASS ORFeome project which aims to establish a collection of TF ORFs (www.grassius.org). These proteins will be used to raise antiserum to be employed in developing chromatin-immunoprecipiation (ChIP) techniques aimed at TF target genes in the maize genome. Thus far the DNA binding domain of one of these (ZmTHX1) was cloned as a His-tag fusion protein in pDEST17 for study of it's preferred binding specificity. We have also identified a Ds element insertion in at least one THX gene and are examining it for possible phenotypes. This project was funded in part by grant NSF DBI-0701405 and IOS-1125620 and the Ohio Plant Biotechnology Consortium.

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P141

Plastid translation mutants and their genetic suppressors in maize

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In maize, mutations of nuclear genes implicated in plastid ribosome assembly and protein translation can be classified into three groups based on phenotype: striped leaf, albino seedling, and embryo lethality. Previous studies of the plastid ribosome deficient leaf striping mutants, *iojap1* (*ij1*) and *striate2* (*sr2*), have revealed evidence of genetic suppressors. Our recent findings indicate that the embryo lethal phenotypes of other plastid translation defective mutants are also suppressed in some inbred backgrounds giving rise to albino seedlings. The *Inhibitor of Striate 1* (*Isr1*) gene, a known suppressor of the leaf striping phenotype of *sr2*, encodes a putative plastid localized hydrolase. Isr1 is proposed to modify striping by inhibiting proliferation of cells in the white sectors of *sr2* mutant leaves. Hence, by studying the suppressors of embryo lethal mutants as well as the *sr2/Isr1* system we may gain insight into the function of plastids in embryogenesis and plant development. In both systems, the suppressor(s) may act by affecting the capacity of plastid defective cells to divide. To understand the molecular mechanism for the formation and suppression of leaf striping, we have characterized natural allelic variation of *Isr1* in diverse maize inbreds and undertaken molecular cloning of the *sr2* gene. A candidate gene for *sr2* identified by bioinformatic analysis of linked genes in the maize genome is shown to encode a putative plastid localized nudix hydrolase homolog 26 (NUDX26). Two tandem copies of *ZmNUDX26* (*ZmNUDX26-1* and *ZmNUDX26-2*) occur in the B73 maize reference genome. Sequence analysis of these genes revealed copy number variation of *ZmNUDX26-2* in the *sr2* mutant, WT, and W22 inbred haplotypes. In addition, genetic analyses are underway to map suppressor(s) of the embryo lethal phenotypes of several different plastid translation mutants. Together these studies will illuminate the relationship if any between *Isr1* and background suppressors of embryo lethal plastid mutants in maize.

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P142

Pullulanase Activity is Associated with Formation of Vitreous Endosperm in Quality Protein Maize

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The *opaque2 (o2)* mutation of maize increases lysine and tryptophan content, but the low seed density and soft texture of this type of mutant are undesirable. Lines with modifiers of the soft kernel phenotype (*mo2*) called “Quality Protein Maize” (QPM) have high lysine and kernel phenotypes similar to normal maize. Prior research indicated that the formation of vitreous endosperm in QPM might involve changes in starch granule structure. Four starch biosynthesis genes, SSIIa, SSIIb, SSIII and Zpu1, have been discovered to have unique alleles in *mo2* lines; therefore these genes may play a role in formation of vitreous endosperm. qPCR analysis of recombinant inbred lines (RILs) derived from a cross of QPM and soft *o2* lines showed a significant increase in expression of the QPM-derived ZpuI allele. Quantitative enzyme activity assays showed that QPM lines had higher pullulanase activity than *o2* and wild type. Furthermore, pullulanase activity was positively correlated with kernel vitreousness in the RILs. Differential scanning calorimetry showed that the thermal properties of starch from the RILs correlated well with the presence of the QPM-derived allele of Zpu1, which had decreased onset and peak endotherm temperatures while total enthalpy of gelatinization was unchanged. Pullulanase activity was negatively correlated with the onset and peak endotherm temperatures but was not correlated with enthalpy. Additionally, pullulanase activity was negatively correlated with the sensitivity of starch granules to digestion by amylase. From these data, we hypothesize that pullulanase is one of the factors that influence amylopectin branch length and crystallinity, which in turn affect the formation of vitreous endosperm in QPM.

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P143

Reverse genetics analysis of the OPPP in maize seed development

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Central carbon metabolism provides precursors for the synthesis of the major storage products in seeds, such as starch, oils and protein. Within central carbon metabolism, the pentose phosphate pathway (PPP) is a major source of reducing power through the production of NADPH. The PPP also produces metabolic intermediates for the synthesis of nucleotides, amino acids, and fatty acids. Recent data from our lab showed that at least one chloroplast-localized PPP enzyme, PGD3, is required for starch accumulation. Interestingly, PGD3 appears to be heat labile, suggesting that the protein can be engineered to improve storage molecule accumulation. We are using a reverse genetics approach to test if additional PPP enzymes are required for maize endosperm development. We have identified 25 loci in the B73 genome that encode the 8 enzymes required for the complete PPP cycle. Nineteen of these genes have been confirmed and annotated by cloning full-length cDNAs. Using UniformMu and MuIllumina transposon Flanking Sequence Tags (FSTs), we have obtained at least one transposon insertion for 20 PPP loci. We are currently screening for homozygous lines for each locus and are generating double, triple, and higher order mutants to reduce or eliminate individual PPP enzymes in the plastid, cytosol, or peroxisome. Subcellular localization predictions are being confirmed or tested using transient expression of GFP fusions with the full length ORFs from the cloned cDNAs. The subcellular localization experiments will be used to guide double and higher order mutant crosses.

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P144

Sequenom-based Bulk Segregation Analysis for Mapping Maize Mutants

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Sequenom-based Bulk Segregation Analysis (Sequenom-BSA) is a unique service offered by the Genomic Technologies Facility (GTF) at Iowa State University. The approach is based on a strategy developed by Sanzhen Liu of the Schnable Lab (Liu et al. 2010 Genetics). A set of 1,016 validated SNP-based genetic markers is used to quantitatively SNP-type pools of mutant and non-mutant siblings from segregating families, thereby genetically mapping the genes responsible for mutant phenotypes. Typically F2 or F1BC progeny are scored for the mutant or wildtype phenotypes and tissue samples from 20-40 (or more) plants are collected per phenotpe, pooled by phenotype, and used for DNA extraction. The two DNA pools are then subjected to a series of multiplexed Sequenom assays. The resulting SNP-typing data are analyzed via a custom bioinformatics pipeline to identify the location of the genes responsible for mutant phenotypes. The BSA service has a mapping success rate of over 95% for single gene mutants. The key determinant for success is the accurate scoring and pooling of mutant and non-mutant individuals.

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P145

Spatial-Temporal RNA Profiling of Early Endosperm Development In Maize

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Maize is the most important agricultural crop in the United States. Maize seeds contain a carbohydrate/protein rich tissue called endosperm that surrounds the developing embryo within the kernel. Two-thirds of all calories consumed by humans come from endosperm directly or indirectly. The carbohydrates and proteins accumulate during late endosperm development and consequently, much research has been devoted to seed physiology during late kernel development. In contrast, little attention has been given to analysis of early endosperm development, which is when the key developmental events (e.g. cell differentiation) occur. Over the last two years, our lab and a collaborating lab initiated a new project to understand how key developmental events in early endosperm are regulated. We initially have focused on understanding the regulation of cell differentiation during this time. We have used laser-capture microdissection (LCM) and RNA-Seq to identify genes expressed specifically in each endosperm cell type. We are currently using reverse genetics approaches to study the functions of the transcription factor (TF) genes that were identified.

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P146

Species independent pharmacologically assisted selection screens, which combine forward genetic approaches with database mining for mutant identification

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Screening of a mutant populations based on phenotype (forward genetic screens) is a common strategy to isolate mutants in currently uncharacterized pathways in crop species of agronomic importance such as maize, sorghum, and rice. The success of such a screen critically depends on the choice of selection criteria, usually based on the assumption of functional conservation. We present a strategy based on classification of mutants by pharmacologically assisted selection screening, which combines pharmacological and classical genetic approaches as well as database mining for genomic data to allow for a more specific and efficient screen. We successfully used this approach to isolate maize mutants with deficiencies in the biosynthetic and perception pathways of phytohormone of the brassinosteroid family. The use of this strategy drastically decreased the number of false positive mutants, and also facilitated the isolation and characterization of novel BR functions in maize. The strategy is species independent and can also be applied to other crops. We show examples of the results of our screening strategy in sorghum, barley and rice.

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P147

Sugarcane mosaic vírus (SCMV) tolerant maize obtained by RNAi

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Corn is one of the most cultivated cereal in the world (155 million ha). Brazil is the third largest producer, behind only the U.S. and China. Among the great losses faced by agriculture in corn are the pests and diseases such as Mosaic (SCMV) and streak virus (MRFV). The effects caused by mosaic in maize plants are greater if the infection occurs earlier, where experiments can show reduction up to 50%. A search for cultivars more productive, disease resistant and adapted to different conditions can be accelerated with the use of techniques such as gene manipulation and transformation. Thus, the purpose of this research is to test the efficiency to develop a tolerance corn to SCMV by expressing the coat protein gene of this virus by the RNAi technology. The coat protein genes of 20 strains isolated from geographic distinct areas around Brazil were sequenced and a 450 bp conserved region was identified. The stability of this sequence was confirmed by Mfold and cloned into the pKannibal vector. The cassette was transferred to pCAMBIA 3301 under the ubiquitin promoter control and transform into the Hi-II maize genotype (sensitive to SCMV). Around 20 events, confirmed by Southern blot analysis, were germinated (4 seeds per event) and tested against the virus. The 15 days old seedlings were inoculated with the virus (carborudum Bioglobal mesh 600) for 4 consecutive weeks, one injection per week. From a total of 57 plants, 16 were asymptomatic. It has also been observed a decrease in symptoms in some of the plants tested in the following weeks after the first infection. Southern blot, PCR and herbicide assays were done to confirm the presence of the transgene and discriminate events. The expression of the gene construction and virus quantification have been followed by Real time PCR. Self pollinated has been done to fix a single copy homozygous transgene in the population to confirm the stability and the real effect of the technology in the field.

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P148

Survey of promoter variation amongst phenylpropanoid pathway genes in maize inbred lines

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Phenylpropanoid biosynthesis, as a crucial metabolism pathway model, is also a well-studied systematic gene regulatory network. The pathway provides a wide variety of secondary metabolites with crucial functions, i.e. sinapoyl esters for UV protection, apymaysins and methoxymaysins for insect resistance, proanthocyanidins for fruit flavor, and lignins for cell wall support. Many of the previous investigations that examine variations in the production of these compounds amongst maize lines, have focused on enzymatic variation and loss of function alleles. In this study we focused on documenting the natural diversity in promoter regions and how those may affect the level of expression of the phenylpropanoid genes. Any natural mutations such as SNPs or indels may alter transcription factor (TF) occupancy by reducing TF affinity, cause improper bending, or altered methylation, and therefore interfere with gene expression. Differential TF occupancy due to cis-allelic variation is also a possible contributor to hybrid vigor but this has not been rigorously tested. As a first step towards understanding the contribution of natural promoter variation to phenylpropanoid pathway gene expression, we have conducted a survey of promoters of over 40 genes in the pathway using the PANZEA database. We report here the frequency of SNPs and indels and the locations relative to known TF target sites. These findings are being used to select suitable germplasm for detection of allele specific occupancy by MYB TFs, and for testing their possible contribution to hybrid vigor. This project is funded by grant NSF IOS-1125620.

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P149

Survey of the Maize Genome for Genes Encoding Autophagy-related Proteins

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A high yield of monocarpic crops depends on not only efficient uptake and assimilation of minerals but also optimal remobilization of assimilated nutrients. Translocation of nitrogen assimilates from senescing leaf cells to sink tissues is preceded by the induction of massive protein degradation pathways including macroautophagy (or simply autophagy), a self-eating mechanism conserved in a variety of eukaryotic cells. We surveyed the B73 maize genome for genes encoding proteins comprising core Autophagy-related (Atg) complexes as well as putative homologues of genes interacting and/or coexpressed with the core *Atg* genes in Arabidopsis. We searched the maize genome browser for possible *Mutator*-insertional alleles and are analyzing them for potential phenotypes such as differential sensitivities to nutritional and environmental stresses. We are also developing strategies to improve nitrogen use efficiency by altering autophagic activity and sink-source relationship in cereals.

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P150

SWEET genes are expressed in the basal endosperm transfer layer (BETL) in developing endosperm of maize

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The basal endosperm transfer layer (BETL) is the first cell layer located at the apo-symplasmic junction between maternal post-phloem region in pedicel and filial generation of developing seeds. BETL is the sole gateway to water and nutrients from the mother plant in maize and is characterized by invaginations of the secondary cell wall with increased surface area and its capacity to transfer nutrients from the apoplasm into the cytoplasm. The most detailed analyses on BETL functions are on its role in sucrose turnover, mainly through the *miniature1* (*mn1*) seed mutation, deficient for the BETL-specific *Mn1*-encoded cell wall invertase (CWI). Specifically, increased levels of sucrose and reduced levels of glucose and fructose in the *mn1* mutant suggest that photosynthates from mother plant may enter the endosperm mainly as sucrose and that the CWI is critical in sucrose hydrolysis in the BETL. How the sugars enter across the plasma membrane into the BETL is unknown. Limited transcriptome studies on BETL did not reveal any clear sugar transporters in these cells (Plant Mol Biol Rep 29:835, 2011); however, there is a highly expressed seven transmembrane domains protein (Zm-seven-TDP) that we show here belonging to the recently discovered family of SWEET sugar transporters (Science 335:207, 2012). SWEETs are membrane proteins highly conserved through most eukaryotes that efflux sugars across cellular membranes. Phylogenetic analyses showed a total of 19 SWEET genes in maize: three of these, *ZmSWEET4a*, *4b* and *4d*, are of special interest because they are nearly identical and clustered together in ~800 kb region on the long arm of chromosome 5, bin 5.04. Further, our *in situ* hybridization analyses show that *ZmSWEET4d* (*Zm-seven-TDP*) has a BETL-specific expression. Detailed spatial and temporal expression analyses of these genes in developing endosperm and functional analyses of the recombinant proteins by Fluorescence Resonance Energy Transfer (FRET) assay are in progress, and will be discussed.

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P151

Systems approaches to understand the role of source-sink relationships in senescence

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Although source-sink relationships play an important role in the regulation of senescence, a degradative process that leads to loss of photosynthetic assimilation in plants, the underlying mechanisms are not completely understood. Our experimental approach to understand regulation of senescence involves removal of the grain sink by preventing pollination and measurement of physiological, transcriptomic, and metabolic. We found that lack of sink in the B73 inbred induces pre-mature senescence due to hyper-accumulation of sugars in the leaf cells and negligible partitioning of sugars to internodes. Thus, it appears that sugar-mediated signaling ("sugar poisoning") in leaf cells is responsible for pre-mature senescence after sink removal. Overlaying transcriptional data from microarrays and RNA-seq onto metabolic networks identified unique features of metabolic flux that are activated upon sink removal including enhanced deposition of structural carbohydrates in the cell wall. We also identified key similarities and interesting transcriptomic differences between induced senescence in maize and natural senescence in Arabidopsis. We screened 450 diverse inbred maize lines and the 250 recombinant inbred lines (RILs) from the intermated B73xMo17 (IBM) population and found substantial natural genetic variation for pre-mature senescence due to sink removal. We identified genotypes that show pre-mature, unaltered, or – surprisingly -- even delayed senescence upon sink removal. QTL analysis of phenotypic data on the IBM RILs together with the availability of induced senescence transcriptome has revealed likely candidate genes that govern onset of early senescence. Our current focus is cloning and characterizing genes underlying endogenous variation for onset of senescence upon sink removal and identification of contrasting genotypes to generate mechanistic insights into source-sink regulation of senescence.

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P152

The collection of maize meiotic mutants illuminates the process of initiation of recombination during the leptotene stage

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The initiation of recombination during meiosis is a critical step that regulates the position of hotspots and crossovers. Meiotic recombination is initiated by the introduction of programmed DNA double strand breaks (DSBs) formed during leptotene by the DNA transesterase SPO11. While the SPO11 protein is well conserved its partners have evolved more rapidly. Maize contains three genes coding for SPO11 as in Arabidopsis. We want to discover and analyze the SPO11 complex in maize. We are taking advantage of the large maize meiotic mutant collection. We have cloned two allelic maize mutations, mtm99-14 and mtm00-03. Both alleles completely delete the SPO11-1 gene. We have characterized two more spo11-1 insertion alleles by reverse genetics. All the maize spo11-1 alleles show meiotic defects that mainly lead to asynapsis and univalent formation. Most of spo11-1 mutants meiocytes show complete absence of DSBs by TUNEL assay and absence of RAD51 foci in the mutant nuclei. However by contrast to what is described in spo11 mutants in other species, around 6% of meiocytes analyzed show residual signs of recombination leading to one to two bivalents. This data suggests a minor SPO11-1 independent DSB formation pathway in maize. In addition to these early recombination defects, cytogenetical analyses show other chromosomal meiotic abnormalities in spo11-1 mutants. These data show a link between the initiation of recombination and axial element conformation predicted but never observed in other species. In order to investigate if this link can be observed in other early recombination maize meiotic mutants, we identified the maize homologs of the known Arabidopsis DSB factors (PRD1, PRD2, PRD3, DFO). We have identify a small deletion in a known meiotic gene in asynaptic (as1), the first maize meiotic mutant discovered [Beadle and McClintock 1928 Science]. Preliminary data suggest that AS1 is also involved in recombination initiation.

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P153

The effect of media substrates on the efficacy of the brassinosteroid biosynthesis inhibitor propiconazole

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Biosynthesis inhibitors are efficient species-independent tools to elucidate hormone functions in plants. Brassinosteroids (BRs) control developmental processes in plants such as: photomorphogenesis, cell elongation, stomatal development, responses to environmental changes, and sex determination. Propiconazole (PCZ) is a widely used fungicide that was characterized as a specific and affordable inhibitor of BR biosynthesis, which allows studying BR function in maize. A disadvantage in working with larger crop species is the inability to use liquid media as a growing substrate. The requirement to use a solid medium to grow maize presents the problem of media interaction with biosynthesis inhibitors like PCZ. To address the possible interaction of PCZ with commonly used growth substrates we developed a test system and effective application method for PCZ in maize. Using plant growth assays and qRT-PCR we show that PCZ efficacy in different substrates, as compared to aeroponics culture, is affected on both levels, growth response and expression of marker genes. FTIR and sorption isotherm analysis determined that vermiculite showed only minimal interaction with PCZ compared to calcined clay substrates, such as Turface, which were able to bind up to 99% of highly concentrated PCZ. Our results suggest that not only the cation exchange capacity (CEC), but also the structure of the growth medium influences the interaction with different hormone biosynthesis inhibitors.
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P154

The identification of regulatory networks that control 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. They have central economical value. 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 genome-wide regulatory network that controls phenolic biosynthesis in maize. For this purpose, we cloned the promoters of 150 phenolic genes into the pDONR-P4P1R entry vector and subsequently subcloned into the pMW#2 and pMW#3 yeast vectors, containing *HIS3* or *LacZ* reporter genes. These promoters will be used as baits in yeast one hybrid assays to screen libraries containing the whole collection (~3,000) of transcription factors responsible for phenolic biosynthesis in maize (TFome). Most of the transcription start sites (TSS) of maize genes remain unknown. Studies in mammals and fungi showed that the majority of genes have more than one TSS. Alternative TSS have profound biological consequences on the gene's function. We performed Cap Analysis Gene Expression (CAGE), a 5' sequence tag approach to determine genome-wide TSS and compose a comprehensive atlas of phenolic regulatory networks in maize. These data will help to identify the cis-regulatory elements that control phenolic biosynthesis in common maize inbred lines, providing functional insights into the regulatory mechanisms of phenolic biosynthesis.

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P155

The Maize Transcription Factor ORFeome (TFome) Project

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Gene regulatory networks are central to all cellular processes. In plants they also help link molecular targets with agronomic traits of functional value including biofuel/biomass production, biomaterials, and nutritional health. An emerging theme is the identification of these regulatory networks in which TFs participate. TFs represent ~7% of the maize genome, consisting of ~3000 genes (~2600 TFs and ~400 Co-regulators). To dissect the gene regulatory network that regulate metabolism of maize phenolic compounds, we initiated The Grass Transcription Factor ORFeome Project (TFome) to clone the entire maize TF repertoire. Full-length ORFs or cDNAs (flcDNAs) are being amplified from existing flcDNAs, genomic DNA, or by RT-PCR, and rare transcripts are custom synthesized. Full-length ORFs (minus stop codons) are amplified and cloned into the Gateway® pENTR/SD entry vector that permits recombination into plasmids for expression in plants or microorganisms. The entire collection is being recombined into yeast two hybrid vectors aimed at finding TFs regulatory partners, combinations of TFs responsible for target regulation of any gene of interest. This approach will contribute to the understanding of metabolic pathways in plants providing a comprehensive TFome collection. Clones for these TFs will be made publicly available through the ABRC at OSU (www.abrc.osu.edu). Information on available clones is being posted at GRASSIUS (www.grassius.org). As part of the database development we have proposed a set of rules for naming TF proteins in the grasses (Gray et al., Plant Physiology 2009 149(1):4-6). This project is currently funded by NSF grant IOS-1125620 and previously by DBI-0701405.

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P156

The molecular genetic dissection of bundle sheath suberization in maize and *Setaria viridis*

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Suberin is a heterogeneous polyester matrix comprised of acyl-lipid-derived aliphatic and phenylpropanoid-derived aromatic components. In grasses, suberized cell walls are found in the endo- and exodermis of primary roots, in wound periderm, and in the bundle (BS) and mestome sheath (MS) layers of leaves. It has been proposed that deposition of a suberin lamella in the C_4 BS cell wall (CW) reduces photorespiration by acting as a barrier to CO_2 escape and O_2 entry from surrounding mesophyll (M) cells. However, none of the underlying biosynthesis or regulatory genes have been characterized in any monocot to date.

We identified a set of candidate genes that are expressed concurrently with sheath suberization in maize, rice, and *Setaria viridis* and assembled a putative biosynthetic pathway based on functional characterizations from Arabidopsis and potato. Comparative transcriptome analyses revealed that suberin, but not cutin, biosynthesis candidates are differentially expressed between C_3 and C_4 grasses. We also identified a set of co-expressed MYB and WRKY transcription factors (TFs) with homology to Arabidopsis TFs expressed in the suberized root endodermis. Promoter sequence analysis of maize suberin biosynthesis candidates revealed canonical AtMYB4 and WRKY binding sites.

To evaluate whether lipophilic CW monomers are comparable between maize and *Setaria viridis*, we developed a protocol to analyze leaf suberin and cutin content. Leaf monomer content is qualitatively similar between the two species; thus, we conclude that *Setaria viridis* is an appropriate model for maize BS suberization. We have disrupted a candidate feruloyl transferase using the maize *Ac*/*Ds* transposons and through an RNAi-based approach in *Setaria viridis* to elucidate the physiological function of BS suberization.

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P157

The role of DCT2 in maize photosynthetic development

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C_4 photosynthesis supports carbon fixation processes that are necessary for the production of food, feed, and bioenergy. The C_4 pathway utilizes the coordinated activities of two distinct specialized leaf cell types, mesophyll (ME) and bundle sheath (BS), to concentrate CO_2 in the BS and minimize photosynthetic losses associated with photorespiration. Chloroplast membrane transporters play an important role in the efficiency of C_4 photosynthesis, by coordinating movement of metabolites between the two cell types. *DCT2* is a maize dicarboxylate transporter that transports malate into BS cell plastids in C_4 plants. The impact of malate transport rates on the overall performance of C_4 photosynthesis in maize, however, has not been characterized. We identified four *Ac* insertions in *DCT2* (two in the first exon, one in the 5' UTR and one in the third intron). Homozygous mutant plants display a yellow leaf phenotype, and suffer severe developmental retardation. These mutants also exhibit a fivefold reduction in their Rubisco content, net photosynthetic rates, and starch accumulation, as well as a 30-50% reduction in Chlorophyll a content, in comparison to wild type plants, suggesting that malate transport into the BS plastids is essential for photosynthetic function in maize. C_4 plants comprise less than 5% of total biomass in the world, therefore the engineering of C_4 features into C_3 crops will enable productivity gains that help feed a rapidly growing global population. We will present recent findings on metabolite transport events that are important to meeting this goal.

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P158

The UniformMu resource: New mutant releases and applications of Mu-Seq

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New mutants and methods for mining them continue to emerge from the UniformMu public resource. This reverse-genetics resource for functional genomics of maize was derived from an inbred population developed specifically for systematic, insertional mutagenesis using Robertson's Mutator transposon. In addition to generating new seed stocks, we have refined our Mu-Seq protocol to enhance identification and mapping of germinal insertions (see also poster by Hunter et al.) with improved accuracy. New mutants have been added to the resource (access via Popcorn at MaizeGDB.org), and together with the upcoming release 6, will bring the UniformMu total to 45,000 independent, germinal Mu insertions. The location of each insertion is precisely defined, and gene-specific flanking sequences are provided. Seeds can be requested on-line, free of charge, through the Maize Genetics Cooperation Stock Center. The resource currently includes over 8,256 seed stocks with insertions including at least 15,000 genes in the filtered gene set. Over 5,000 genes have two or more insertion alleles. Tools for accessing the resource and tips for analyzing insertions in genes of interest will be presented.

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P159

Tissue-specific regulation of maize zein genes

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Differentiation of multicellular organism requires a combination of trans-acting factors that regulate their target genes at multiple levels, of which transcriptional regulation is the first step in gene expression. Whereas some target genes that perform general functions are constitutively expressed, others are spatially and temporally regulated, only expressed at certain time and tissue. Such an example is the major storage proteins in *Zea mays*, which are alcohol-soluble prolamins, called zeins that are exclusively expressed in endosperm 10 days after pollination. Their tissue specificity is conferred by the actions of several endosperm-specific transcription factors and the epigenetic modifications of their DNA-binding sites. Most zein-gene promoters contain a conserved cis-acting element, called prolamin-box (P-box), recognized by the trans-activator P-box Binding Factor (PBF). Because of the lack of null mutants, its physiological role in storage-protein gene expression has been elusive. In contrast, a null mutant of another endosperm-specific trans-activator Opaque2 (O2) is required for the transcriptional activation of subsets of this superfamily by binding to the O2 box. Therefore, we used RNA interference (RNAi) to knock down *Pbf* expression and found that only 27-kDa γ- and 22-kDa α-zein gene expression was affected, whereas the level of other zeins remained unchanged. Still, transgenic seeds had an opaque seed phenotype. Combination of *PbfRNAi* and *o2* resulted in further reduction of α-zein expression and a more severe opaque phenotype. We also tested the interaction of promoters and constitutively expressed PBF and O2 in leaves. Whereas transgenic promoters could be activated, endogenous promoters appeared to be not accessible to transcriptional activation, presumably due to modified chromatin states. Although analysis of the methylation of binding sites of PBF and O2 correlated with the expression of endogenous 22-kDa α-zein promoters, a different mechanism seems to apply to the 27-kDa γ-promoter, which does not undergo methylation changes.

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P160

Trans-activation of *zein* genes in oat-maize addition lines

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Oat-maize addition (OMA) lines are oat plants with maize chromosomes in addition to their own. Previously, we showed that the zein regulators *Opaque2* and *Pbf* are expressed in immature endosperms of OMA lines in the presence of single maize chromosomes 7 and 2, respectively. In addition, the 27- and 50-kDa *gamma zeins*, which are located on OMA line with maize chromosome 7, are also expressed in the same tissue. Given that *Pbf* is absent on the said OMA line, and the fact that it is needed for expression of the 27-kDa *gamma zein*, we hypothesized that PBF function should be conserved between oat and maize. We therefore set out to clone the oat *Pbf* (*ASPbf*) gene and test whether it can trans-activate the expression of 27- and 50-kDa *gamma zeins*. PCR primers were designed from *Pbf* of maize (*ZMPbf*) and wheat (*TAPbf*), and were used to amplify *Pbf* in oat using genomic DNA. PCR products were obtained from primers designed from *TAPbf*, while no amplification was observed from primers designed from *ZMPbf*. The PCR products derived from *TAPbf* primers were then sequenced and analyzed. One of the sequences (993 bp long) turned out to have 71% sequence identity with the *TAPbf*, and further analysis revealed that it had a predicted zf-Dof domain – a defining characteristic of *Pbf* genes. RT-PCR of mRNA extracted from immature endosperm using primers designed from the putative *ASPbf* gene showed a fragment around 1 kb in agarose gel electrophoresis, indicating the oat gene consists of a single exon. Analysis of the predicted protein sequence of this gene showed that it has 67.4% sequence identity with *TAPbf* protein, including the zf-Dof domain. Therefore, it appears that a basic regulatory function has been conserved across two different subfamilies of the grasses.

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P161

Transcriptional analysis of head smut resistance in maize: how to resist the early infection and late proliferation of Sporisorium reilianum f. sp. zeae

(submitted by Yonglian Zheng)

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The head smut fungus in maize, Sporisorium reilianum f. sp. zeae, which is an important biotrophic pathogen responsible for extensive crop losses, infects maize by invading the root during the early seedling stage. Head smut is not obvious until the tassels and ears emerge. S. reilianum has a very long life cycle that spans almost the entire developmental program of maize after the pathogen successfully invades the root.
In order to investigate disease-resistance mechanisms at this early seedling stage, digital gene expression (DGE) analysis, which applies a dual-enzyme approach, was used to identify the transcriptional changes in the roots of Huangzao4 (susceptible) and Mo17 (resistant) after root inoculation with S. reilianum. During the infection in the roots, the expression pattern of pathogenesis-related (PR) genes in Huangzao4 and Mo17 were significantly differentially regulated at different infection stages. The glutathione S-transferase (GST) enzyme activity and reactive oxygen species (ROS) levels also showed changes before and after inoculation. The total lignin contents and the pattern of lignin depositions in the roots differed during root colonization of Huangzao4 and Mo17.
In order to understand how this pathogen interacts with the host during its long life cycle at the molecular level, and how this interaction differs between susceptible and resistant varieties of maize after hyphal invasion, the maize 70mer-oligonucleotide microarrays were used to investigated transcriptional changes in the resistant maize line Mo17 at four developmental stages. We found that there was a lengthy compatible relationship between the pathogen and host until the early 8th-leaf stage. The resistance in Mo17 relied on the assignment of auxin and regulation of flavonoids in the early floral primordium during the early floral transition stage. We propose a model describing the putative mechanism of head smut resistance in Mo17 during floral transition. In the model, the synergistic regulations among auxin, flavonoids, and hyphal growth play a key role in maintaining compatibility with S. reilianum in the resistant maize line.

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P162

Virus-Induced Gene Silencing in Diverse Maize Lines Using the Brome Mosaic Virus-based silencing vector

(submitted by Peter Balint-Kurti <peter_balintkurti@ncsu.edu>)

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Virus-induced gene silencing (VIGS) is a widely used tool for gene function studies in many plant species, though its use in cereals has been limited. In addition, within cereal species the varieties that best respond during VIGS screens are often not known. Using a Brome mosaic virus (BMV) vector designed to silence the maize phytoene desaturase (PDS) gene, a genetically diverse set of maize inbred lines was screened for development of gene silencing after inoculation of seeds through the novel use of a vascular puncture inoculation technique. In addition to Va35, which previously was shown to support silencing, maize lines NC300, Ki11, Oh7b, M162W and CML52 displayed significant visible photobleaching when challenged with the BMV-PDS. In these plants, targeted PDS mRNA expression was decreased 50-80% relative to levels in plants that were inoculated with BMV containing a fragment of the GUS gene or were mock-inoculated.

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P163

What's adult plant resistance got to do with host metabolism in maize?

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Adult plant resistance (APR) is an important form of resistance in plants; however how it operates remains unknown. The maize – CCR1 pathosystem promises a unique opportunity to fill this void. This interaction is characterized by the disease Northern leaf spot (NLS), key agent of which is HC-toxin. The *Hm1* maize disease resistance gene confers immunity to NLS by encoding an NADPH dependent HC-toxin reductase (HCTR), which inactivates HC-toxin. Resistance conferred by *Hm1* is absolute and operates in every part of the plant. In contrast, *Hm1A* (an allele of *Hm1*) and *Hm2* (a duplicate of *Hm1*), confer effective protection only at maturity. Cloning of these APR genes revealed that while *Hm1A* encodes an HCTR with five amino acid substitutions, *Hm2* encodes a truncated HCTR lacking the last 52 amino acids of HM1. These structural changes perhaps only weaken the HCTR function of their corresponding peptides and not kill their activity completely. Targeted mutagenesis of *Hm1* with EMS was used to genetically validate this hypothesis. This procedure led to the generation of two new APR alleles of *Hm1*. Since these APR alleles were the result of two relatively conserved amino acid substitutions, T90M and V210M, respectively, it clearly shows that partial loss-of-function mutations of *Hm* gene lead to alleles with an APR phenotype. The question then is why do such weak alleles behave in an APR fashion? One possibility is that the level of NADPH, which appears to fluctuate widely in maize seedlings, falls below the threshold required for the mutant HCTR enzymes encoded by the APR *Hm1* alleles. Given that *Hm1A* seedlings become more resistant to CCR1 when grown in the presence of 3% sucrose or under extended light conditions, seem to validate our hypothesis.

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P164

ZmCCT10 is a negative regulator of flowering time controlling photoperiod sensitivity in tropical maize

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A major photoperiod QTL on chromosome 10 (Ducrocq et al., 2009; Coles et al.,2010) was identified as a CCT domain (CONSTANS, CONSTANS-LIKE, TOC1) protein and named ZmCCT (Hung et al., 2012). We independently cloned the same gene based on homology to rice Ghd7 (stands for grain number, plant height and heading date 7), which is a floral repressor under long days in rice. The CCT domain proteins form a large family in maize. To discriminate this gene from others we assigned a number, ZmCCT10, according to its chromosome location. We investigated the diurnal expression of ZmCCT10 in the day length neutral temperate B73 and the day length sensitive tropical line CML436. In the temperate line ZmCCT10 transcription is low regardless of the day length. In the tropical line under the short days, ZmCCT10 is also expressed at low levels. However under long days ZmCCT10 showed a strong diurnal pattern with the peak of expression at 10 a.m. Moreover, the amplitude of the peak under long days was 10 folds higher than that under short days. Expression pattern of the floral activator ZCN8 was in the opposite phase compared to ZmCCT10. These patterns suggest that ZmCCT10 functions as the negative regulator of the floral transition in photoperiod sensitive lines by targeting directly or indirectly ZCN8.

P165

Whole transcriptome profiling of maize inbred line A188 during early somatic embryogenesis reveals altered expression of stress factors and embryogenesis-related genes

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The ability to form embryogenic cultures and regenerate green plants in vitro is a critical factor in plant genetic engineering. Maize regeneration ability is genotype-dependent and limiting to transformation-based genomics. Research was undertaken to examine the transcriptional profile in the early stages of culture initiation from immature embryos of the highly embryogenic and regenerable maize genotype A188. Gene expression levels in immature embryos collected at different time points (0, 24, 36, 48 and 72 hours after plating in culture) were analyzed via RNA-Seq analysis. Out of 39,456 B73 5b reference genes, 25,241 were expressed. Genes annotated as stress factors accounted for a large portion of genes with altered expression: 16.7% of all down-regulated and 10.8% of all up-regulated genes during the first 24 hours. Stress factors induce somatic embryogenesis which is a necessary precursor to regeneration ability. Several transcription factors were also altered in expression. AP2 (APETALA 2) and EREBP (ethylene-responsive element binding protein) families represented 12.66% and 8.14% of transcription factors that were up-regulated and down-regulated, respectively. More specifically, embryogenesis-related transcription factors, such as maize BABY BOOM 1 and BABY BOOM 2, which are similar in sequence to the AP2/ERF family of transcription factors, were altered in expression. These genes were previously described to promote cell proliferation and spontaneous formation of somatic embryos. The maize LEAFY COTYLEDON 1 gene, also shown to be involved in induction of somatic embryogenesis and PINFORMED1, which mediates auxin flux triggering patterning and differentiation, showed multi-fold expression changes over the time course of this experiment. In addition, SOMATIC EMBRYOGENESIS RECEPTOR KINASE genes, which maintain and modulate embryogenic competence in culture, were expressed at a steady state. In summary, this study provides information on genes expressed during early embryogenesis that may play a role in somatic embryo formation in maize and other plant species.

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P166

Water-responsive maize leaves: leaf rolling potential of bulliform and bulliform-like cells

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WARTY2 (*Wty2*) encodes a receptor-like tyr kinase that regulates epidermal patterning in maize. Mutant *wty2* homozygotes show altered expansion and position of epidermal cells that are histochemically similar to bulliform cells. Normally, bulliform cells are thought to initiate leaf rolling during drought stress to protect maize leaves from water loss. Over-abundance and misplacement of *wty2* bulliform-like cells to the abaxial epidermis cause excessive leaf rolling in the mutant suggesting specific regulation of bulliform cell expansion or placement by the kinase. Preliminary observations suggest mutant leaves respond more rapidly than non-mutant to both dehydration and rehydration, based on an *in vitro* leaf rolling assay. To test potential responsiveness to varied water availability, a precision controlled irrigation experiment was designed that would test for physiological responses in the leaf rolling mutants compared to non-mutants. Stomatal conductance (g), CO_2 uptake (A) and transpiration (T) were compared under three irrigation regimes of 0.50ETc, 0.75ETc and 1.00ETc (based on reference evapotranspiration and a crop-specific coefficient and representing varying water supplied from drought to optimal conditions respectively). In general, the mutant showed higher rates or A, g, and T than the non-mutant, especially under chronic drought stress. A preliminary explanation for these results is that the mutant was less water stressed due to increased leaf rolling, especially under the lowest ETc regime. Yield differences were not measured in the preliminary plot. A full field experiment will be conducted to test further the potential impact of mutant and non-mutant leaf rolling on physiological parameters associated with water use.

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P167

Alternative splicing of *Rgh3* transcripts regulates protein abundance in the spliceosome

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Alternative RNA splicing produces multiple mRNA species from individual genes increasing protein diversity and regulating gene expression. Genome sequencing projects have shown that about 42% to 45% of intron-containing genes in plants are alternatively spliced, but little is known about how alternative splicing is controlled. The *rough endosperm3 (rgh3)* mutant causes developmental defects that are either seed or seedling lethal. *Rgh3* encodes a U2AF35 related protein (URP), which is a predicted RNA splicing factor. U2AF35 proteins identify splice acceptor sites during RNA processing and function through protein-protein interactions by creating complexes with U2AF65 and other Serine/Arginine rich-proteins. Semi-quantitative RT-PCR analyses of alternatively spliced genes showed that rgh3 affects splicing in a subset of genes supporting a role for RGH3 in alternative splicing. *Rgh3* is alternatively spliced, producing at least 19 different spliced variants. Interestingly, only one variant is predicted to encode a full-length URP ortholog containing an N-terminal acidic domain followed by two zinc fingers flanking a UHM domain and a C-terminal RS-like domain. Several Rgh3 splice variants produce truncated proteins missing one to several domains. GFP fused to full-length RGH3 localized to the nucleolus and nuclear speckles. Moreover, GFP fused to the endogenous truncated protein variants showed that while the acidic domain contains a nuclear localization signal, the RS-like domain enhances nuclear localization and is also important for protein recruitment to nuclear speckles. The UHM domain is a modified RRM domain that allows protein-protein interaction and in RGH3 it enables co-localization with U2AF65. Bi-molecular fluorescence complementation assays proved that U2AF65 interacts with U2AF35 as well as with RGH3. These results indicate that RGH3 participates in the U2-type spliceosome and suggest that its function is regulated by alternative splicing by creating truncated protein variants that are excluded from the spliceosome.

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P168

Analysis of cell fate acquisition in maize anthers by high-throughput small RNA profiling

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Unlike animals, plants lack a germline. Only after floral somatic organs exist do cells differentiate as microsporocytes (precursors to sperm) and megasporocytes (precursors to eggs). It is largely unknown how meiotic cell fate and the switch from mitosis to meiosis are initiated. Maize anthers are a superb system to address these questions because of their large size and simple anatomy, which makes it possible to access stages and cell types more readily than in other plants. We sequenced small RNAs of carefully staged maize anthers and found that small interfering RNAs of either 21nt or 24nt are preferentially expressed in a phased manner at different developmental stages. In parallel with this molecular approach, a genetic screen was performed on maize UniformMu lines that harbor known mutations in genes involved in small RNA production and activity. Candidate genes that are required for meiotic cell fate acquisition will be investigated to study the function of phased small RNAs in maize.

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P169

Analysis of signals coordinating tissue identity during leaf development

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The semi-dominant *Hairy Sheath Frayed1* (*Hsf1*) mutations occur in one of the maize cytokinin receptor proteins, Zea mays Histidine Kinase1 (ZmHK1), causing inappropriate cytokinin signaling during early leaf development. As a result, *Hsf1* mutant plants have altered proximal-distal leaf patterning; projections, or prongs, of proximal sheath, auricle and ligule tissue extend from the distal leaf blade margin. To gain insight into how and when inappropriate cytokinin signaling establishes prong initiation, we undertook a detailed morphological analysis of prong formation. We measured the distance from the ligule to the position of each prong on mature leaves. Analysis of prong position along the leaf blade shows that there are specific areas where prongs are more or less likely to occur, indicating prong formation is not a random event. In fact, prong position in the *Hsf1* mutant background reveals putative sub-compartments within the leaf blade. Further, we are investigating the stages of leaf development at which prong formation begins, as well as how prong growth coordinates with the normal regions of cell division and cell expansion. The timing and location of prong appearance will be compared with developmental hallmarks, such as appearance of the ligule. These data are expected to provide meaningful insight into how signals coordinate within the leaf to maintain correct tissue identity during development.

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P170

Branch meristem initiation is dependent on the activities of the functionally redundant SBP box transcription factors unbranched2 and unbranched3

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The maize inflorescence produces several different lateral meristems with different activities. One of these, the branch meristem (BM), is necessary for production of the agronomically important long branches found at the base of the tassel. The presence of long tassel branches allows for extended pollen shedding as well as an increase in the amount of pollen, two factors crucial for proper seed set. We recently discovered that mutations in members of the tasselsheath 4 (tsh4) clade of SBP box transcription factors display a strong decrease in the number of BM made by the tassel. A reverse genetic screen performed with genes similar to tsh4 uncovered two duplicate loci, unbranched2 (ub2) and unbranched3 (ub3), which abolish BM initiation in double mutant combinations. Single ub2 or ub3 mutants only show a modest decrease in BM initiation, while double mutants lack BM. This BM phenotype is enhanced by the presence of the tsh4 mutation. An antibody was raised to the UB2 and 3 proteins and used for immunolocalization. Despite their sequence similarity to tsh4, both UB proteins occupy separate domains within the tassel compared to TSH4. Interestingly, neither UB protein is found within the BM despite being necessary for BM initiation. Within the shoot apex, UB is found at the base of the meristem, in leaf primordia and the stem, but not in the tip of the meristem. We hypothesize that ub2 and ub3 are functionally redundant factors necessary for controlling cell partitioning during BM initiation. When ub2 and ub3 are mutated, cells are allocated to other parts of the phytomer such as leaves and stems instead of the meristem, resulting in fewer cells being available for BM initiation.

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P171

Characterization and cloning of *Barren inflorescence3*, a novel semi-dominant maize mutant

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Maize inflorescences include the uppermost male tassel and several lateral female ears, all of which are formed from groups of pluripotent stem-cells called meristems. Meristem initiation and activity are established through the coordinated regulation of different genes and hormones that are required to balance the maintenance of the stem-cell population and the formation of lateral organs. Several previously characterized maize *barren* mutants, affected in inflorescence development, have highlighted the fundamental role of the plant hormone auxin in the formation and activity of meristems. As part of a collaborative effort aimed at unraveling the role of auxin in maize development, we are characterizing a novel semi-dominant maize mutant, called *Barren inflorescence3 (Bif3)*. Defects in the *bif3* gene function result in the formation of a shortened central spike in the tassel and of short bald ears. Detailed morphological analysis of heterozygous *Bif3* mutant inflorescences shows a significant decrease in the number of paired spikelets that are formed, and close observation indicates that these pairs are instead replaced by single spikelets or regions completely devoid of spikelets. Furthermore, the inflorescence meristem often appears to collapse during development. These phenotypes suggest that *bif3* is required for the initiation and maintenance of meristem activity. Using a map-based cloning approach we narrowed the region containing the *bif3* locus to 0.23 Mb on chromosome 2, and we are currently pursuing possible candidate genes in this window. Double-mutant analyses with other semi-dominant *barren* mutants, *Barren Inflorescence1 (Bif1)*, *Barren Inflorescence4 (Bif4)*, and known mutants required for auxin biosynthesis and response, *sparse inflorescence1 (spi1)* and *barren stalk1 (ba1)*, respectively, are also underway to study potential interactions among these genes.

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P172

Characterization of a New Developmental Mutant of Maize: rld*5409

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In maize, the *rolled leaf1* (*rld1*) and *rolled leaf2* (*rld2*) genes encode HD-ZIPIII proteins that accumulate on the adaxial side of developing leaves. *rld1* and *rld2* are involved in establishing abaxial/adaxial leaf polarity and in regulating *yabby* gene expression. The expression domain of *rld1* and *rld2* is controlled by *microRNA166* (*miR166*), which targets *HD-ZIPIII* transcripts for degradation and thereby limits their expression domain to cells on the adaxial side of the leaf. Dominant *miR166*-insensitive alleles that lead to misregulation of *rld1* result in plants that exhibit an upward rolling of leaves along their margin, as well as ectopic ligules and macrohairs on the abaxial leaf surface. A new, recessive rld-like mutant, designated *rld*5409*, displays a similar leaf phenotype. We examined juvenile and adult leaves using various histological techniques. On *rld*5409* leaves we observed a reduction of macrohair and bulliform cells on the adaxial epidermis, regions of the abaxial epidermis containing macrohairs, and altered stomatal patterning on both adaxial and abaxial leaf surfaces. Vascular tissue polarity appeared normal. These observations suggest that the *rld*5409* mutant has some adaxial/abaxial epidermal patterning defects. Expression of genes in the *miR166/HD-ZIPIII* pathway (*miR166b, ago1, rld1, rld2, zyb9, zyb14* and *zyb15*) was examined in *rld*5409* and wild-type leaf primordia, juvenile and transition leaf tissues, as well as developing ear tissue, by quantitative RT-PCR; however, no significant differences were observed. Expression of genes involved in asymmetric cell division (*dcd1, add1, tan1, pan1* and *pan2*) was also examined using quantitative RT-PCR with cDNA prepared from the basal 1 cm of leaves harvested from 14 day old plants; no statistical difference was determined between *rld*5409* and wild-type samples. Current studies include mapping the location of the gene responsible for the *rld*5409* mutant phenotype.

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P173

Characterization of a novel *barren* mutant in maize

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All aerial structures in plants are determined by the activity of meristems, groups of undifferentiated stem cells. Maize inflorescences, the tassel and the ear, and the spikelets and flowers they carry, are produced by a series of specialized meristems, called axillary meristems. In maize, mutants affected in the formation of axillary meristems are generally referred to as *barren.* These mutants are usually characterized by having fewer branches and flowers in both the tassel and the ear. Our goal is to isolate and characterize new *barren* mutants and identify the affected genes to increase our understanding of the molecular mechanisms and pathways required for maize inflorescence development. The recessive mutant *barren inflorescence 173* (*bif173*) was originally identified in an EMS mutagenesis screen targeting mutants affected in the formation of axillary meristems. *bif173* mutants are characterized by defective inflorescence development, such as a reduction in the number of spikelets and branches in the tassel and smaller and more disorganized ears. These phenotypes are reminescent of mutations affecting the biosynthesis, transport or signaling of the plant hormone auxin. Bulk segregant analysis showed that the *bif173* locus was linked to chromosome 8, between bins 8.04 and 8.05. This analysis confirmed that *bif173* represents a novel maize *barren* mutant. In order to clone the corresponding gene we are carring out a fine mapping approach with SSR, indel, dCAPS and CAPS markers on an F2 segregating population, obtained by crossing *bif173* to the inbred line B73. Although the mutant phenotype is not fully penetrant, we have successfully assigned the *bif173* locus to a small 1.2Mb window. We are now exploring this region for possible candidate genes. To unequivocally identify the *bif173* gene we are also pursuing an alternative approach based on RNA-seq analysis.

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P174

Chromatin dynamics of the *knotted1* locus during shoot development

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Dynamic and precise control of gene expression during development is crucial for multicellular organisms. It has been shown that the regulation of chromatin status such as epigenetic modifications and physical interactions with enhancers play an important role in animal development. However, regulatory mechanisms of developmental regulators at the chromatin level are poorly understood in plants. *knotted1* (*kn1*), which encodes a homeobox transcription factor, is highly expressed in the shoot apical meristem (SAM), and is tightly silenced in leaves. *kn1* is essential for SAM maintenance, and its silencing is required for proper leaf development. To understand the mechanisms of chromatin regulation of *kn1*, we investigated epigenetic histone modifications by ChIP assay. In wild type B73, the active mark H3K4me3 accumulated at the 5' region of the gene in shoot apex tissue, and was rapidly removed in leaf tissue. A repressive mark H3K27me3 was detected in the entire gene at low level in the shoot apex, and increased through the leaf development. In the *Kn1-N* dominant allele, which has an *rDt* transposon insertion in intron 4, H3K27me3 accumulation was almost completely lost, and ectopic accumulation of H3K4me3 was observed at the *rDt* insertion site. Similar results were observed in other dominant alleles. We also investigated DNA methylation in B73, *Kn1-N* and an epigenetic gene silencing mutant of RNA-dependent RNA polymerase *mop1*. In B73, some cytosines in exon2 were methylated in leaf blade, but not in shoot apex. This methylation is decreased in *Kn1-N* and in *mop1*, suggesting a potential role of DNA methylation on *kn1* silencing. Finally, our previous observations suggest the presence of distal regulatory elements of *kn1* and we aim to test this possibility by chromatin physical interaction studies.

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P175

Cytological and morphometric analysis of *Zea mays* early endosperm

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The development of cereal endosperm has been well characterized in barley; however, descriptions of maize endosperm development are incomplete. A comprehensive staging of maize early endosperm in line B73 is vital for future work describing the gene networks controlling endosperm development and function. We present cytological and morphometric descriptions of maize endosperm development, from fertilization to a differentiated endosperm prior to the accumulation of storage products. The development of the endosperm is described using semi-thick sections of plastic embedded kernels (0-8 DAP) and confocal microscopy. We divide development into stages; coenocytic (subdivided into 3 stages), cellularization (subdivided into 6 stages), and differentiation with 5 distinct tissues. Maize endosperm develops through 2-4 rounds of alveolation followed by a random division of the remaining central vacuole. This contrasts with barley that cellularizes completely through repeated alveolation. A morphometric comparison of development between Arizona and Michigan grown B73 shows a common pattern of development. However, AZ endosperm starts to develop 1 day earlier than MI endosperm. By 4 DAP, AZ endosperms are 2 days more advanced in cytology and size and this 2 day difference continues through 12 DAP. The comparison of B73 development with NAM lines may lead to correlations between early stage endosperm development and mature kernel characteristics.

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P176

Defining the regulatory networks controlling inflorescence architecture in maize

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Branching patterns of grass inflorescences are determined by position and developmental fate of stem cell populations called meristems. Genetic control of branching is a primary determinant of yield, regulating seed number and harvesting ability, yet the molecular mechanisms underlying inflorescence architecture in grasses remain largely unexplored. Here, using developing maize ear and tassel primordia as our model system, we defined distinct developmental modules that contribute to the identity and determinacy of grass-specific meristem types. Our integrative approach associated molecular phenotypes (ie. developmentally-staged mRNA-seq expression profiles) with spatiotemporal changes in morphology during development and response to genetic perturbation. The latter includes loss-of-function mutations in the *ramosa* (*ra*) genes, key regulators of a pathway controlling branching. *ra1* and *ra2*, which encode transcription factors (TFs), and *ra3*, a sugar metabolic enzyme, are essential for repressing branches. We found that genes known to work together in other developmental contexts are reused in modules for spikelet pair and spikelet meristem development, along with uncharacterized and/or grass-specific genes.

To expand our co-expression network, we generated genome-wide occupancy maps for RA1 by performing ChIP-seq with transgenic RA1-tagged lines. Data from two independent constructs were compared from developing ear and tassel primordia. Our results showed that RA1 acts, either directly or indirectly, to activate or repress genes during development and that the mechanism of RA1 action depends on spatiotemporal context. Bound and modulated targets of RA1 included known developmental genes as well as uncharacterized TFs, and key genes that integrate multiple hormone and signaling networks. We also investigated potential combinatorial binding for shared targets between RA1 and KNOTTED1 (KN1), a master regulator of meristem maintenance, using published KN1 ChIP-seq data. Genes co-targeted by RA1 and KN1 were enriched for TFs, which were co-expressed in distinct spatiotemporal contexts, and suggest points of regulation that interface meristem maintenance and determinacy

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P177

DICER-LIKE4 plays a key role in ta-siRNA biogenesis and exhibits functional redundancy with *DICER-LIKE1*

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Leaf polarity along the adaxial/abaxial (top/bottom) axis is governed by a complex network of interactions between transcription factors, miRNAs and other small regulatory RNAs. The ta-siRNA pathway participates in this regulation and generates a 21nt class of small RNAs, termed ta-siARFs, which are adaxially localized and act antagonistically toward *auxin response factor 3* (*arf3*) genes. Mutations in one of the biogenesis components of the pathway, *dicer-like4* (*dcl4*), condition a leaf polarity phenotype that is partially characterized by sectors of abaxial identity on the adaxial leaf surface and/or by radialization of the leaves. We show that the ta-siRNA pathway in *dcl4* mutants is compromised in the production of 21 nt ta-siRNAs and that this translates into a spatial redistribution and accumulation of its target transcripts, the *arf3* genes. Expression of a non-targeted version of *arf3a* is able to recapitulate many of the developmental defects observed in *dcl4* mutants. Surprisingly, we find that a null allele of *dcl4* conditions a relatively mild phenotype, whereas mutants in other ta-siRNA pathway components exhibit more severe developmental defects, consistent with functional overlap between *dcl4* and other maize *dicer* genes. To address this issue, we are utilizing a viable partial loss-of-function *dicer-like1* allele (*dcl1-2*) and also a *dcl2* allele that contains a Mu insertion in its 5' UTR. The *dcl1-2* mutant displays some features of the *dcl4*, *lbl1-rgd1* and *rgd2* ta-siRNA pathway mutants, including a reduction in overall plant stature, as well as impaired mediolateral expansion of the leaf blade and sheath. *dcl4 dcl1* double mutants exhibit an enhanced phenotype whereby the embryos are developmentally compromised and kernels fail to germinate. Combined with the fact that 21 nt ta-siARFs are partially retained in the *dcl4* null allele, these findings suggest a redundancy within the maize dicers that is not observed in other species.

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P178

Double mutant analysis of the GRAS family transcription factor *upright leaf angle1 (url1)*

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An increase of the leaf inclination angle in maize has been observed in elite hybrid lines since the 1970s (1). Using an integrated pharmacological, genetic, and bioinformatics approach we isolated *url1* with a putative phytohormone deficient phenotype in maize. *url1* and its monocot orthologs cluster in a novel clade of the GRAS-transcription factor superfamily. Mutants of *url1* produced semi-dwarf statures with shorter but wider, dark green and crinkled leaves. In addition, *url1* leaf angles showed a drastically increased leaf lamina inclination angle of upper leaves. Distinct from the classical ligueless mutations *lg1* and *lg2*, which are implicated with leaf angle control in maize (1), *url1* leaves showed no severe defects of the leaf collar region. Multiple lines of evidence indicate that *url1* acts as a monocot specific, positive regulator of phytohormone signaling.

Expression profiling suggests that *url1* mRNA accumulates primarily in tissues with high meristematic activity and to a lower extent at the base of developing leaves. RNA-seq analysis identified 129 differential expressed genes in ligule tissue and 234 differential expressed genes in meristem and leaf tissue of *url1* compared to wild type controls. Double mutant analysis between *url1* and the brassinosteroid biosynthesis mutant *na1* showed an increase in penetrance of tasselseed formation as compared to *na1* single mutants. Double mutants of *url1* and the GA biosynthesis mutant *d5* exhibited a significant decrease in tiller formation compared to *d5* single mutants. Scanning electron microscopy of double mutants showed an increased aberration in cell arrangement compared to WT or single mutants.

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P179

Dynamics of phase-specific patterns of differentiation in maize leaves.

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Vegetative phase change in maize is accompanied by morphological change in leaves including the appearance of adaxial trichomes and the diminished presence of cuticle wax. We are investigating the spatiotemporal development of these morphological changes with respect to the age of the leaf: we want to know how early or late in the development of a leaf do these phase-specific traits first appear, and whether the timing is the same for each successive leaf on a plant. The first 4 leaves in the line we study are invariably morphologically juvenile (trichomes absent, wax present); the next 2 to 3 are transitional, mosaic for juvenile and adult traits. All leaves after the eighth have adult morphology (trichomes abundant, wax scarce). The age, based on plastochron index, is a key parameter in understanding this process. The timing of appearance of phase-specific traits will be used to interpret results of gene expression studies ongoing in the lab that investigate the genetics of vegetative phase change.

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P180

Ecophysiological characterization and grain yield of two maize hybrids contrasting to drought

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In Brazil, North of Minas Gerais State, the lack of rainfall limits crop production at the field, and cropping at certain times is only possible with irrigation. Agronomic practices and physiological characteristics have been heavily sought to overcome the drought and consequently increase production. Within this context, the objective of this study was to characterize the morphophysiological changes and evaluate the attributes of grain yield in two maize hybrids contrasting to drought under field conditions. The experiment was carried out for two years and drought stress was imposed by suspending irrigation for 22 days at pre-flowering. At the end of stress it were assessed the following characteristics: chlorophyll content, percentage of dry leaves, leaf area, stomatal conductance, chlorophyll fluorescence, Anthesis Silking Interval (ASI) and yield components. For better interpretation of tolerance traits, an index was used (Relative Value of Tolerance, RVT). The hybrid DKB 390 (tolerant) overcame BRS 1030 (sensitive) in grain production. Besides that, it showed a lower percentage of dry leaves, short ASI, higher stomatal conductance and higher Fv/Fm. We conclude that significant morphophysiological changes have occurred related to drought tolerance in DKB 390, which resulted in increased productivity in the field.

Keywords: Zea mays L., water stress, stomatal conductance, harvest index.

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P181

Engineering apomixis in *Zea mays* L.

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Apomixis is defined as asexual reproduction through seed. Despite this phenomenon was reported in over 400 species it is absent in major crops. The production of seeds genetically identical to their mother has a high agricultural potential to maintain desired genotypes indefinitely, e.g. the maintenance of heterozygosity in hybrids.

Gametophytic apomixis deviates from sexual development in three major steps: apomeiosis (lack of meiosis leading to the formation of unreduced, unrecombined egg cells), parthenogenesis (activation of embryogenesis without fertilization of the egg cell) and functional endosperm formation. Our approach is to search 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 screens for mutants displaying apomeiosis and parthenogenesis. The genetic screen for apomeiosis mutants was based on the ploidy barrier of the endosperm. Plants carrying highly active *Mu* transposons were pollinated by 4n^*R-nj* and the progeny was screened for plump kernels displaying R-nj pigmentation at the scutellum and aleurone. Screen resulted in 3 mutants that showed development of viable diploid egg cell with different frequency. Ploidy of the progeny was assessed by flow cytometry and the maintenance of maternal heterozygosity was evaluated by SSR analysis. Confocal microscopy confirmed the absence of normal meiotic divisions in the mutants.

A second screen for mutants displaying parthenogenesis was performed by pollinating $bz1^{mum9}$ lines with 2n^*R-nj*. The absence of R-nj pigmentation in the embryo is an indication for possible parthenogenetic development of the haploid embryo. Putative haploid kernels were germinated and their ploidy was assessed by flow cytometry. Confirmed haploids were treated with colchicine to induce diploidization and will be pollinated by 2n^*R-nj* to confirm the genetic basis of the observed phenotype. Promising parthenogenetic mutants will be analyzed by molecular and cytological means.

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P182

Essential role of a sucrose phosphate phosphatase gene in maize embryo and endosperm development

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Grain filling is a critical biological determinant of crop yield. We aim to identify genes that are essential for grain filling by adapting MuSeq, a novel NexGen-sequencing based genotyping method, to perform co-segregation analysis of a large number of transposon tagged seed mutants. Using this approach, we identified sucrose phosphate phosphatase 1 (*Spp1*) as an important grain filling gene. SPP catalyzes the last step of sucrose biosynthesis, hydrolysis of sucrose-6-phosphate into sucrose. A Mutator transposon insertion in the *Spp1* locus, *spp1-umu1*, causes a shrunken kernel phenotype, reminiscent of *shrunken-1* seeds. To examine function of *Spp1* in development of embryo and endosperm, we first compared overall morphology of wild type and mutant seed tissues during development. Consistent with the shrunken kernel phenotype, endosperms of nearly mature *spp1* mutant seeds have an internal cavity suggesting that endosperm filling is incomplete. Surprisingly, however, dry weight of mutant kernels did not differ significantly from wild type. In addition, total starch content of the *spp1* mutant endosperm appears to be unaffected. Fresh weights of wild type and *spp1* mutant embryos were also comparable at the seed maturity. However, development of the *spp1* mutant embryos was slightly delayed compared to wild type during early seed maturation. SPP activity is thought to be essential for plant growth. Maize B73 genome has two genes, *Spp1* and *Spp2*, indicating possible functional redundancy. Both genes are broadly expressed in maize tissues including embryo and endosperm, though *Spp1* expression is typically greater than *Spp2*. Mutator insertion allele of *spp2*, *spp2-umu1*, did not show a discernible phenotype. The double mutant is under investigation.

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P183

Evalutation of the role of IBA-derived IAA in maize development

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Auxin is a phytohormone involved in cell elongation and division. In monocots, auxin regulates the development of adventitious and lateral roots, vascular tissues, leaf number, and leaf blade area. Because of the importance of auxin, levels of indole-3-acetic acid (IAA), the primary auxin, are tightly regulated through biosynthesis, degradation, sequestration, and transport. IAA is sequestered in reversible processes by adding amino acids or sugars, forming IAA-conjugates, methylation, or via a two carbon elongation forming indole-3-butyric acid (IBA). These sequestered forms of IAA reduce the ability of the hormone to act as a signal. Regulation is controlled in individual tissues and developmental stages and at a subcellular level. For example, in Arabidopsis ILR1, which hydrolyzes IAA-L and IAA-F to free IAA, is expressed primarily in two day old cotyledons, whereas IBA-response genes are most highly expressed in early seedling development. Organelle location also separates auxin pools in the plant. IBA metabolism occurs in the peroxisome whereas IAA-amino acids are hydrolyzed in the endoplasmic reticulum. The many ways of regulation indicate the importance of maintaining specific amounts of IAA in the plant. For example, the ill2iar3ilr1ibr1 quadruple mutant has delayed germination and shorter root hairs.

We are translating the current knowledge of IBA-derived IAA from Arabidopsis to maize. In Arabidopsis, IBA to IAA conversion occurs in a multistep process and involves the enzymes IBR1, IBR3, IBR10, and ECH2. We have identified maize genes similar to AtECH2, AtIBR1, AtIBR3, and AtIBR10 with 78, 82, 80, and 68% identity. These genes are conserved among many plant species all retaining the peroxisome targeting signal. Preliminary studies show mutations in ZmECH2, ZmIBR3, and ZmIBR10 cause phenotypes including random embryo placement and decreased tassel branching. Elucidating the role of IBA-derived IAA in maize will lead to increased understanding of auxin homeostasis in maize.

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P184

Evolution of the bract suppression network: exaptation or de novo integration?

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Vegetative growth in most plants is dominated by leaf development. However, upon transition to reproductive development, leaf growth is often completely inhibited. Leaves that grow in an inflorescence are called bracts, and bract suppression is common in many plants including the model systems maize (*Zea mays*) and Arabidopsis (*Arabidopsis thaliana*). We have undertaken a genetic approach to identify the gene network that regulates bract suppression in maize. Previously we isolated the *tassel sheath1* (*tsh1*) and *tsh4* genes from mutants that fail to suppress bract growth. Both genes encode transcription factors expressed in the cells of the suppressed bract primordium. Interestingly, orthologs of *tsh1* and *tsh4* have no role in bract suppression in Arabidopsis, suggesting that distinct genetic mechanisms were recruited for bract suppression in these two lineages. In order to identify more components of the maize bract suppression pathway we have performed a screen for enhancers of the *tsh1* mutant phenotype. Over ten novel *enhancer of tsh1* (*ent*) mutants have been identified to date. These mutants have diverse phenotypes, and many have pleiotropic phenotypes consistent with aberrant hormone signaling. These results suggests that bract suppression is regulated by a complex genetic network including hormonal pathways. Since the grass family convergently evolved bract suppression, future work to understand the bract suppression network may shed light on an interesting aspect of developmental evolution, namely when "novel" genetic pathways evolve, are they co-opted as a unit from previous functions, or are novel networks integrated de novo?

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P185

Examining the phenotypes of three developmental mutants in diverse genetic backgrounds

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Genetic modifiers are typically identified by enhancer/suppressor screens via mutagenesis. Simultaneously, breeders and geneticists increasingly acknowledge the importance of favorable alleles derived from natural variation. Combining both ideas, we are screening for genetic modifiers by crossing *Clumped tassel1* (*Clt1*), *Few branched1* (*Fbr1*) and *Liguleless3-O* (*Lg3-O*), to the diverse inbreds that are the NAM founders and 5 ex-PVP lines.

Clt1 reduces the lengths of the tassel rachis, branches and internodes. Furthermore, ear size and plant height are also decreased. We are in the process of cloning this gene and have narrowed its location to a 6Mb interval on Chromosome 8L. This region contains 27 candidate genes supported by public cDNA and EST data, of which several are promising candidates we are sequencing. In addition, we have identified a putative enhancer of *Clt1* among our diverse lines. Data will be presented for one putative enhancer of *Clt1* that produces very small (~40cm) plants with well-spaced internodes and fertile tassels and ears.

Fbr1 reduces the number of tassel branches and encourages the growth of bracts. In the F2 of crosses to Mo18w, plants with *Fbr1* phenotypes occurred less than expected ($P<0.001$) and bracts were observed in a substantial number of plants with wildtype branch numbers. We hypothesize that Mo18w carries a suppressor of the reduced branching phenotype though not the bract formation phenotype and are genotyping to test this idea and to begin understanding this apparent separation-of-function.

Lg3-O mutants have upright leaves due to displaced ligules. While no *Lg3-O* modifiers have been identified yet, we have now sequenced the *Lg3-O* allele. Compared to B73, we found multiple SNPs, insertions and deletions, but no changes to the predicted amino acid sequence. However, we can now genotype *Lg3-O* plants by amplifying polymorphic regions within *lg3*, which will aid in identifying suppressors.

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P186

Exploring the role of auxin in ligule development: Reporter expression in maize ligular region explants

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The ligular region in the maize leaf consists of a ligule fringe and a superjacent wedge of auricle. These structures define the boundary between the blade and sheath and contribute to blade angle, thereby regulating an important agricultural trait. We seek to understand when and how this important structural boundary in the leaf is first established. A preligule band of rapidly dividing cells is the first visible structure followed by lateral outgrowth of the ligule fringe, but the signals to trigger the site specification are not known. Recent work by Moon, Hake and others (2012) shows localization of auxin-associated markers at the ligule site. To understand the potential mechanism of auxin action at the boundary, we have taken three approaches: (1) imaging currently available reporters for auxin at the ligule site including PIN1-YFP and DR5, which are both proxies for auxin transport and presence respectively; (2) developing a maize semi in-vivo explant system for pharmacological experiments. The system is specifically used here to test for the role of auxin and auxin inhibitors on reporter expression; and (3) adapting a new reporter protein that degrades in response to auxin (Degron, reported by Brunoud et al. 2012) and tested its efficacy in explants via transient expression. Results show PIN-1 localizes specifically to the ligule in a non-polarized pattern, based on fluorescence quantification around the cell periphery. DR5 expression is generally low in the ligule, but can be induced by auxin treatment in the explant system. We hypothesize that PIN1 may exclude auxin from the site, but further observations require more reliable auxin reporters in maize. Preliminary evidence suggests the new Degron reporter functions as expected in maize. Further experiments are ongoing and a stable transgenic maize line expressing the Degron is being produced.

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P187

Expression of Transcription Factor Genes in Early Endosperm Development in Maize

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Our long-term goal is to understand the gene networks controlling endosperm development and function in maize. In flowering plants, endosperm provides nutrients and signals to the embryo during seed development or the seedling during germination. In cereal grains, endosperm comprises a large proportion of the mature seed and contains large amounts of carbohydrates and proteins. During early seed development in maize, the endosperm undergoes many rounds of mitosis without cytokinesis, forming a multi-nucleate structure (syncytial phase), becomes fully cellularized (cellularization phase), and then undergoes a period of intense mitosis (early mitotic phase) to produce the bulk of the mature seed. The molecular processes controlling these processes have not been elucidated. To create a framework for understanding the regulatory networks controlling maize endosperm development, we have used a combination of mRNA-Seq and laser-capture microdissection to identify the spatio-temporal patterns of gene expression during early endosperm development. These assays have identified many transcription factor genes expressed during early maize endosperm development. We have selected a subset of these genes for functional analysis in maize plants.

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P188

Expression Patterns and Interactions of Developmental Genes in Maize

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The shoot apical meristem (SAM) is a dome-like structure containing stem cells responsible for producing all of the above-ground organs of the plant. Its dual functions of organogenesis and stem cell maintenance require a complex network of genetic control. Multiple gene families are expressed in the SAM and have been implicated in meristematic identity and function. Much of what we know about the role and architecture of the SAM and the growth and development of maize in general comes from the analysis of mutants. However, less is known about the genes controlling the wide range of natural variation in gene expression and SAM architecture that exists across different genetic backgrounds in maize. Using published RNA-seq datasets, we examined expression and interaction of genes implicated in development and leaf formation (eg KNOX or YABBY) across different inbreds, tissues, and developmental stages. We also investigated gene expression in the Intermated B73 x Mo17 Recombinant Inbred Lines (IBMRIL) using RNA-seq data from shoot apices; these data were combined with eQTL analysis to elucidate gene interactions acting in the SAM. Finally, measurements of SAM architecture were utilized to reveal correlations among gene expression and the genetic control of size and shape in the maize SAM.

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P189

Functional characterization of members of the maize (*Zea mays* L.) Aux/IAA gene family

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In maize, 31 Aux/IAA genes have been identified (*Wang et al*. 2010). All of them share four characteristic domains. Domain I functions as a transcriptional repressor and domain III and IV are known to control homo and heterodimerization with other Aux/IAA genes and/ or Auxin Response Factors (ARF). Domain II with the conserved degron-sequence GWPPV is responsible for the stability of Aux/IAA proteins. In general, Aux/IAA proteins are localized in the nucleus.

Comprehensive expression studies of different maize root and shoot tissues revealed unique expression patterns for members of the Aux/IAA family. Thus far only one Aux/IAA mutant is known in maize. *Rootless with undetectable meristem 1* (*rum1*) has a 26 amino acid deletion in domain II (*von Behrens et al*. 2011) and does neither show any lateral roots on the primary root nor any seminal roots compared to the wild-type. Characteristic point mutations in the degron-sequence are sufficient to stabilize the affected Aux/IAA proteins and thus confer a mutant phenotype. This has been previously demonstrated for different Aux/IAA mutants like *bodenlos* (*bdp*) (*Hamann et al*.1999) or *short hypocotyl2* (*shy2*) (*Tian et al*. 1999) in *Arabidopsis thaliana*. Based on the results of the expression studies, we plan to generate novel Aux/IAA mutants in maize. Candidate genes were mutated in the degron-sequence and protein stability and subcellular localization studies were performed. Furthermore, interaction studies are in progress.

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P190

Genetic analysis of spontaneous double haploid in maize (Zea mays L.)

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Double haploid (DH) lines has gradually become a key technology in maize (Zea mays L.) research and breeding nd exploiting the haploids' natural fertility may enhance efficiency of line development. Here we used shedding traits (performance in exposed anther and pollen) to describe characteristics of the proportion of haploid fertility (FP) exactly. Nine inbreds haploid derived from different heterotic groups were evaluated for FP in two different places and high parent (8701) and low parent (Zheng58) were found, which were composed of haploid population to do QTL mapping of haploid fertility making use of segregation distortion. Four QTL have been detected in chromosome 1, 3, 4 and 10. Meanwhile, super high parent (100% FP) and super low parent (0% FP) fertile haploid were also selected from biparental population composed by both low parents Zheng58 and Chang7-2 (both were less than 50% FP), which means haploid fertility was controlled by multiple minor effects interacted genes. Flow cytometry identified three different mixoploid-types of double haploid correponding with different shedding ratio in a haploid population, which means somatic cell double and gamete cell double were independent spontaneous double process of each other.

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Genetic and Biochemical Analysis of *Hairy Sheath Frayed1* (*Hsf1*) Function

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Maize leaves are composed of four specific tissue types organized in a distinctive pattern along a leaf's proximal-distal axis. The sheath is proximally located, the blade is distal while the auricle and ligule tissues form the boundary in between. *Hairy sheath frayed1* (*Hsf1*), a semi-dominant gain of function mutant, disrupts this proximal-distal organization. *Hsf1* mutants exhibit tissue outgrowths on the distal blade margin consisting of the typically proximal auricle, ligule and sheath tissues. The gene underlying the *Hsf1* phenotype is *Zea maize His-kinase1* (*ZmHK1*), one of the seven cytokinin receptor histidine kinases found in maize. Sequence analysis of three *Hsf1* alleles shows single nucleotide missense mutations in the cytokinin (CK) binding domain of the receptor. The *Hsf1* mutant ZmHK1 receptors have altered CK binding affinities and signal in the absence of CK in heterologous histidine kinase signaling assays. We performed structural modeling of the CK binding domain of ZmHK1 using the published 3-D structure of the arabidopsis Histidine Kinase4 (AHK4) protein. Our analyses show that all three *Hsf1* alleles create critical amino acid substitutions near a loop that forms part of the CK binding pocket. These results allowed us to hypothesize that each *Hsf1* missense mutation disrupts specific amino acid interactions that alter the position of the loop, thus changing the conformation of the CK binding pocket. Notably, the structural modeling indicates that Glu236 forms an H-bond with Arg192 in the loop. This bond is disrupted by the *Hsf1-1603* mutation - a Glu236Lys change. We are using targeted mutagenesis of specific residues near the CK binding pocket and the heterologous histidine kinase signaling assay to test this hypothesis, and investigate other molecular interactions that may affect CK binding and signaling. Results of these tests will be presented, along with our molecular characterization of transposon insertion alleles of the *ZmHK1* gene.

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P192

Genetic architecture of meristem morphology in diverse maize inbreds and the genus Zea

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Through combined organogenesis and maintenance of stem cell identity, the maize shoot apical meristem gives rise to all above-ground tissues found in the adult maize plant. Unlike the aerial tissues it produces, the shoot apical meristem of maize is difficult to observe during vegetative growth. Traditional methods of whole-meristem dissection or serial sectioning require high levels of skill, labor, and biological replication to accurately measure and examine intact shoot apical meristems. These limitations have slowed our understanding of maize meristem morphology and how it is correlated to important adult plant traits. By combining mathematical modeling of maize meristems as parabaloids and sensitive optical and x-ray observation (nanoCT) techniques, we have developed a semi-automated, higher-throughput phenotyping platform for characterizing morphological phenotypes in the shoot apical meristems of diverse maize lineages. Using detailed meristem phenotype data from diverse lines of agricultural importance, we will examine the correlation between meristem morphology and adult plant traits of agronomic importance. Future work aims to couple our phenotyping platform to linkage mapping, comparative transcriptomics, and functional analysis of candidate genes to explore and understand the genetic architecture and developmental regulation of this critical stem cell niche.

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P193

Genetic improvement in ear development

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Since the widely-adopted introduction of maize hybrids in the late 1930's, the beginning of the hybrid era, grain yield of maize has steadily increased by roughly 1 kg $ha^{-1}yr^{-1}$. Density tolerance is primarily responsible for the yield improvement achieved during the hybrid era. Some progress has been made toward identifying the physiological mechanisms that confer density tolerance such as improved dry matter accumulation and growth rate around silking. However taken together, neither mechanism completely explains the density tolerance of modern hybrids. Recent studies demonstrate that distal spikelets which form late in ear development exhibit atypical morphology and do not contribute to the final kernel number. We hypothesize that the genetic improvement of increased density tolerance is due to changes in spikelet development. To examine this hypothesis we will use five Canadian ERA hybrids that reflect genetic improvement over five decades. Comparison of the atypical spikelet ratio and the density tolerance for each hybrid will reveal whether spikelet development underlies the genetic improvement to the final kernel number per ear. Using three densities, potential and actual yield will be evaluated at one location for two years. This research will provide novel insight into maize yield potential and will identify the next "biological weakness" for maize breeders to target.

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P194

Histological and Molecular Characterization of Maize Mutant *rgd-378*

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The trans-acting small interfering RNA (ta-siRNA) pathway is involved in the establishment of dorsiventral polarity and mediolateral expansion in plant leaves. Mutations that disrupt this pathway in maize, as observed in *raggedseedling2* (*rgd2*) and *leafbladeless1* (*lbl1*) plants, lead to dramatic alterations in leaf shape. A new developmental maize mutant, *rgd-378*, with phenotypic characteristics similar to *rgd2* and *lbl1* has been identified. *rgd-378* mutants are small plants with variable leaf abnormalities including bifurcated leaves, filamentous leaves, and leaves that fail to expand mediolaterally on one or both sides of the midrib. In some *rgd-378* leaves, mediolateral expansion is lost on the proximal portion of the blade, but not at the distal end. Juvenile and adult *rgd-378* leaves were examined using various histological techniques. Epidermal impressions were prepared from juvenile and adult stage leaves of *rgd-378* plants and compared to wild-type; *rgd-378* leaves displayed defects in epidermal patterning, which included altered stomatal patterning, smaller pavement cells, and mild alterations in subsidiary cell shape due to apparent subsidiary mother cell division failure. Quantitative RT-PCR was used to compare expression of genes involved in the ta-siRNA pathway (*tas3a, miR390a, miR390b, rgd-2, lbl1, dcl4, rdr6* and *arf3a*) on cDNA prepared from *rgd-378* and wild-type leaf primordia; no differences in expression of these genes were detected. An interval on chromosome 10 was identified by bulked segregant analyses to be the likely location of the gene responsible for the *rgd-378* phenotype; we are examining candidate genes in this region.

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P195

Identification and mapping of branching modifiers in ramosa mutants

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ramosa1 encodes a C2H2 zinc finger protein and likely transcriptional repressor and is a key regulator in a pathway that limits the growth of lateral branches in maize inflorescences. Tassels of *ramosa1* mutants display increased branching and ears exhibit unorganized rows and/or long branches. The severity of the *ramosa1* mutant phenotype varies significantly with genetic background. For example, the phenotypes of *ramosa1* mutant alleles are stronger in B73 than Mo17 inbred backgrounds. Using the IBM population, we exploited these phenotypic differences to map modifiers of ear branch number, with a goal to identify additional ramosa pathway members. Each RIL in the IBM-94 population was backcrossed to Mo17- and B73-introgressed *ra1-63.3359* to generate two F1BC1 populations. In each population, ear branch number was used to map modifiers of branching in the B73 and Mo17 backgrounds. We identified several loci in Mo17 and B73 including one large effect QTL on chromosome arm 1S, which alone accounted for approximately one third of the total phenotypic variance. Recombinant lines were generated from a near-isogenic line (NIL) that segregated for the 1S modifier region. By using a progeny testing approach with recombinants, we are now focusing on ~250kb within which we are testing candidate genes. We employed a similar strategy to map modifiers of *ramosa2*, which encodes a LATERAL ORGAN BOUNDARY (LOB) domain transcription factor and interacts genetically with *ra1*. *ramosa2* mutants also display increased inflorescence branching and similar background effects in B73 and Mo17. Using IBM-94 we identified three major QTL, one of which maps to the same region as the *ra1-63.3359* modifier. Simulations showed that QTL mapping results would have varied greatly with data from less than ~85 IBM lines. Utilizing our NILs and a subset of those produced by the Springer lab, we are generating recombinants to narrow down the *ra2* modifier locus intervals.

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P196

Identification of novel gene networks involved in stem cell maintenance and organogenesis in maize

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The shoot apical meristem (SAM) initiates all above-ground organs and is maintained through the activity of a population of stem cells. The gene regulatory networks controlling meristem indeterminacy and organogenesis remain largely unknown. To gain insight into these networks, we generated a high-resolution gene expression atlas for the shoot apex. We combined laser microdissection and RNA sequencing to analyze key functional domains, such as the stem cell-containing SAM tip, the newly initiating leaf (P0), developing leaf primordia (P1-P3), the epidermal L1, the underlying L2, and vasculature. This comprehensive data set allows us to identify genes predicted to function in distinct meristem and leaf patterning processes. CAST clustering identified co-regulated genes that specifically mark the whole meristem, its stem cell domain, the P0, or developing leaf primordia. Furthermore, previous work has shown that a number of key genes regulating meristem functions are targets of small RNAs. To specifically address the role of small RNAs in SAM function and organogenesis, we obtained small RNA profiles for the shoot apex by small RNA deep sequencing, and established expression patterns of mature miRNAs by *in situ* hybridization. We further resolved the expression profiles of the miRNA precursor genes using our high-resolution atlas. These analyses revealed distinct mature miRNA expression patterns, suggesting diverse contributions of miRNAs and the pathways they target in SAM function. Generally precursor and mature miRNA expression correlated well. However these comparisons also revealed unexpected discrepancies in the accumulation of certain mature miRNAs and their corresponding precursors in specific cell types. The finding suggests complex regulation of miRNA biogenesis, stability and mobility within the SAM. Characterization of the expression patterns of validated miRNA targets is still ongoing and will provide information about miRNA action. Finally, this rich dataset will be used to direct functional studies of meristem indeterminacy and organogenesis.

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P197

Investigating reproductive isolation between maize and teosinte

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Crossing between maize and some strains of teosinte is unilateral, in that teosinte pollen fertilizes maize when hand pollinated, but fertilization of teosinte by maize fails. We aim to clone the genes that confer this barrier and understand the underlying mechanisms. *In vivo* pollen tube growth assays show that maize pollen tubes are arrested in teosinte silks. This barrier is governed by a single locus *tcb1* (*teosinte crossing barrier1*). The *Tcb1-s* haplotype present in tesosinte encodes a female factor that blocks maize pollen (haplotype *tcb1*) and a male factor that overcomes that block. We created a mapping population of 16,451 chromosomes after crossing a *Tcb1-s* teosinte strain to a W22 inbred line. Using comparative mapping to the maize B73 reference genome, gene expression analysis, and sequencing, we pinpointed the male factor to a gene that is highly expressed in pollen. The teosinte allele of this candidate gene has elevated expression compared to the maize allele and two amino acid changes in the coding region. cDNA of this gene from teosinte driven by a maize pollen-specific promoter has been cloned and is being transformed to maize to confirm its function. The female factor was mapped to a neighboring region that contains four protein coding genes on the B73 genome. We sequenced BACs from *Tcb1-s* genome covering the mapping region. Using transposon-tagging, we also created a mutant line in which the female gene is knocked out, but the male gene function is intact. Cloning these genes will help to understand reproductive isolation, polar cell growth and cell-cell interaction. On a broad sense, this study will provide scientific basis to regulate gene flow between populations and thus enhance agriculture and ecology outcomes.

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P198

Investigating the role of boron transport during maize inflorescence development

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Boron is an essential micronutrient for plant growth and development, playing an important role in the structure and maintenance of plant cell walls. In maize and other crop species, boron is also important for obtaining high and good quality yield. We have isolated a recessive maize mutant, *rotten ear* (*rte*) that shows impaired development of maize inflorescences, the tassel and the ear. Strong alleles of *rte* produce non viable pollen grains and ears that appear to rot and decay early in development. Positional cloning of *rte* revealed that it encodes a boron efflux transporter, and heterologous complementation confirmed that it is a functional homolog of the *Arabidopsis thaliana BOR1* gene. In *Arabidopsis* roots, BOR1 is responsible for the loading of boron in the xylem. Accordingly, *RTE mRNA* is also localized in the vasculature in the cells surrounding the xylem in vegetative and reproductive tissues. The RTE protein contains ten putative transmembrane domains and subcellular localization studies confirm that it is present at the plasma membrane. In maize a close duplicate gene, *RTE-LIKE1*, exists and expression analysis suggests that its mRNA expression pattern overlaps with *RTE*'s. We have identified transposon insertions in the *RTE-LIKE1* gene and we are now investigating if, similarly to what we described in *rte* mutants, defects are also observed in the formation or fertility of maize inflorescences. Double *rte;rte-like1* mutants are under construction to understand the role of both genes in the uptake and distribution of boron during maize development.

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P199

Is the Year Effect on Grain Yield Related to Proper Female Floret Development?

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In maize, grain yield is highly related to kernel number. There are numerous factors, including pollination and kernel abortion that influence final kernel number. However, there is very little evidence to suggest that the total number of florets initiated varies year to year in the same way as kernel number. Recent work in our lab and by others suggests that not all florets develop properly and that under less favorable environments, such as high plant density, a higher proportion of florets in the distal portion of the ears will not develop enough to allow pollination to occur. When underdeveloped florets are controlled for, the number of normally developed florets equals the number of kernels on an ear. We hypothesize that proper floret development is one of the underlying causes of the year effects in modern maize germplasm. We will use a set of 4 inbred lines and 5 hybrid lines at 3 planting densities (40,000 plants ha^{-1}, 80,000 plants ha^{-1} and 160,000 plants ha^{-1}) grown for 3 years (2011-2013). This will allow us to observe ear development and grain yield with an approach that accounts for differences in photosynthate availability to determine the effects of density on kernel development. This research will help elucidate the physiological basis of the year effect on grain yield.

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P200

Maize Cell Genomics: Developing a two component transactivation system

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Analysis of maize at a systems level is now possible with advances in functional genomics tools. New methods are still needed to interrogate protein function experimentally and at the subcellular level. To this end, we have generated over 100 stable, natively expressed, fluorescent protein (FP) fusion lines that mark all common subcellular compartments. These lines thus far have provided new views of maize subcellular organization, identified novel promoters, and provided a useful molecular resource for the maize community. We are currently developing an LhG4 2-component transactivation expression system to drive cell, tissue and organ-specific expression. Selected promoters are used to activate expression of the maize-codon optimized LhG4 transcription factor, which in turn will transactivate genes of interest driven by the pOp promoter. Drivers currently being constructed include meristem-specific promoter and upstream regulatory regions from WUS and RA3 and leaf-specific promoters from LGL1, and WTY2. Others currently in the pipeline include ZmSUT1, ZmMDH, ZmOCL6 and ZmRAB2A. Once experimentally tested and confirmed, our project will deliver to the research community stable transformants of 50 promoter/driver lines, as well as 20 new FP tagged lines and new methods for live cell imaging of meristems and leaves. Experimental results, images and metadata will be available and processed using Bisque, an imaging database management and analysis system and accessible to the maize community via our project website. We welcome new requests from the maize community through our website, http://maize.jcvi.org/cellgenomics/index.php.

Contact Dave Jackson or Anne Sylvester for more information.

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P201

Mapping of EMS- and NAM-founder- derived modifiers of mutants that affects inflorescence structure in maize.

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To elucidate the mechanism of genes controlling inflorescence architecture traits, we took a genetic approach to identify second site modifiers of two classical maize mutants: *fasciated ear 2 (fea2)* and *ramosa3 (ra3)*. The maize mutants in *FEA2*, a *CLAVATA2* homolog, make fasciated ears and thick tassels due to an enlarged inflorescence meristem. Mutants in *RA3*, which encodes a sugar metabolism related enzyme, trehalose-6-phosphate phosphatase, make branched ears and more branched tassels due to loss of spikelet pair meristem determinancy.

We found and characterized some EMS-induced enhancers of *ra3*. As a complementary approach, we also identified natural modifiers coming from the diverse NAM (Nested Association Mapping) founder inbreds by screening F2 populations of the mutants crossed to NAM founder inbreds. We found a strong enhancer of *fea2*coming from the NC350 inbred, of *ra3* coming from the Ki11 inbred, and a suppressor of *ra3* coming from Mo17 inbred. Seggregation ratios suggest one recessive locus for the NC350 derived enhancer, one semi-dominant locus for the Mo17 derived suppressor, and two or more loci for the Ki11 derived enhancer. Initial results of bulk segregation analysis of the modifiers as well as approaches to fine mapping the modifier loci will be presented and discussed.

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P202

Maternal gametophyte effects on maize seed development

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Angiosperm seeds arise from double fertilization of the embryo sac by the two sperm cells of the pollen grain. A variety of molecular mechanisms are used to ensure that the events in seed development occur in the appropriate time and space. In addition to gene activity from the zygotic genome after fertilization, there are also gene activities specific to the maternal gametes, their supporting cells, or the maternal alleles in the endosperm that are essential for normal embryo and endosperm development. To understand the biological processes under maternal control in the seed we have performed a large-scale screen for maternal effect mutants producing abnormal but viable seeds. Analysis of these mutants reveals maternal gametophyte effects on endosperm patterning, seed growth efficiency, and embryo development. These mutants fall into four classes of pre-fertilization phenotypes. Additionally, several of the mutants have reduced male transmission indicating a separate requirement for the gene in pollen function. The architecture of the central cell, as revealed by the position of the polar nuclei, is important for patterning of the endosperm. Additionally, analysis of mutants with reduced antipodal cell cluster size is consistent with a role for antipodal cells in seed development.

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P203

MicroRNA function in maize development

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MicroRNAs (miRNAs) are small non-coding RNAs that repress gene expression in all multicellular organisms and function in diverse developmental and physiological processes in both plants and animals. Most plant miRNAs promote cleavage and degradation of mRNA targets, resulting in decreased expression of miRNA target genes. We have isolated a maize mutant, fuzzy tassel (fzt), which contains a mutation in the RNase III domain of DICER-LIKE 1, a key enzyme required for miRNA biogenesis in plants. fzt plants have severe phenotypic defects affecting both vegetative and inflorescence development. In the inflorescence, all meristem types are less determinate than normal, and florets do not make normal floral organs, resulting in both male and female sterility. fzt mutants also have severe vegetative defects including reduced plant height, narrow leaves, and defects in known miRNA-regulated processes including phase change and leaf polarity. To understand how the fzt mutant affects miRNA levels in vivo, we examined the small RNA populations from whole fzt and normal seedlings using deep sequencing analysis. Most miRNAs are moderately reduced in fzt seedlings, however a few miRNAs are more dramatically reduced. We are currently examining the small RNA populations and transcriptomes of tassel primordia in fzt and normal plants. We will choose select miRNA target genes misregulated in fzt mutants for further investigation, including RNA in situ hybridization and mutant analysis. Our ultimate goal is to use the fzt mutant as a tool to identify key miRNA and mRNA target pairs required for specific stages of development.

P204

Modifier mapping, and expression analysis using RNAseq in the dominant *Liguleless Narrow* mutant

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Changes in the development of leaves in maize can have drastic impacts on the viability of the entire plant. We are interested in understanding the genes which regulate leaf development. Heterozygous *Liguleless narrow* mutants have shorter, narrower leaves, however in some Inbred lines the heterozygous mutants significantly recover. Learning what kind of gene is causing the recovery could also shed light on the developmental process of leaf formation in the grasses. Monitoring differential gene expression in an expected region can lead to localizing a new gene faster than by traditional mapping alone. Performing this investigation will also reveal other genes that may be affected by this developmental pathway making future research faster and better directed. Identification of which gene is causing the recovery could have impacts on the ability to produce hardy crops.

Along with traditional mapping of recombinants, RNAseq data from the four genotypes using Illumina sequencing will identify genes in the vicinity of the modifier that are good candidates based on differential expression. By selecting tissue from the shoots and roots for plants with and without the modifier, and with and without *lgn*, a profile of gene expression based on genotype will be created. Roots and shoots were studied based on previous expression data for sister of liguleless narrow (sln) a homolog for lgn, and the fact that many developmental genes are deferentially expressed between the shoots and roots.

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P205

Molecular Genetic Dissection of Auxin in Maize Embryo Sac Development

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The female gametophyte is central to successful fertilization and seed production. In angiosperms, the female gametophyte has four cell types: the egg cell, synergids, central cell and antipodal cells arranged along the micropylar-chalazal axis. Using the expression of fluorescent reporters driven by DR5 or Pin1 promoters as a proxy for the presence of auxin maxima, auxin is restricted to the antipodal cell cluster in maize embryo sacs in contrast to the micropylar end seen in Arabidopsis. One model for auxin function in maize and other cereals is a proliferative function in the antipodal cells, in contrast to Arabidopsis antipodal cells, which degenerate during embryo sac development. A number of maize mutants with altered antipodal cell morphology are being characterized to explore their effects on auxin patterns. One of these, *indeterminate gametophyte1 (ig1)*, encodes a LATERAL ORGAN BOUNDARIES domain protein with high similarity to ASYMMETRIC LEAVES2 of *Arabidopsis thaliana*. Genes potentially regulated by auxin and ig1 in the embryo sac were identified by a search of genes misregulated in ig1 mutant embryo sacs for the presense of cis Auxin Response Elements (AuxRE) . In-situ's with Maize Auxin Response Factors (ZmARF's) support the action of auxin in maize antipodal cells. The *Lax-midrib1-O (Lxm1-O)* mutant, which has reduced or delayed antipodal cell proliferation, was examined for effects on post-fertilization seed development. Fertilization of Lxm1-O embryo sacs exhibit a development lag in endosperm development.

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P206

Morphoanatomy of roots for two maize hybrids contrasting to drought tolerance

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A large number of studies has been carried out throughout the world in order to evaluate the effect of climate changes on agriculture. The variation in soil water availability is one of the major factors in climate change. Within this context, the objective of this study was to characterize root morphoanatomic changes in two maize hybrids contrasting to drought under field conditions. The water deficit was imposed by suspending irrigation for 22 days at pre-flowering growth stage. The anatomic evaluations were performed by the end of stress period and for better interpretation of tolerance traits in the hybrids evaluated, (DKB 390 – tolerant and BRS 1030 – sensitive), an index was used (Relative Value of Tolerance, RVT). The DKB 390 resulted in a greater amount of aerenchyma in root cortex, increased exodermis thickness, metaxilemas in a greater number and smaller diameter. We conclude that significant root morphoanatomic changes have occurred related to drought tolerance in DKB 390 which possibly facilitated its survival and better performance at field conditions.

Keywords: Zea mays L., water stress, root anatomy, aerenchyma

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P207

New *tassel-less* mutants with defects in vegetative and reproductive development in maize

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Tassel-less mutants are characterized by an absent or reduced tassel and ear and vegetative defects. At least eight *tls* loci have been identified and mapped and two have been cloned. Here we present the mapping data and characterization of the *tls4* and *tls5* mutants. *tls4* and *tls5* mutants produce narrower leaves and are shorter than normal due to the production of shorter internodes. *tls4* and *tls5* mutants have defects in both vegetative and reproductive development. *tls4* and *tls5* tassels have a decrease or complete lack of reproductive organs. Tassel branch and spikelet quantification of *tls5* mutants demonstrates that the mutants produce fewer branches and spikelet pairs, with more single spikelets than normal siblings. SEM analysis of *tls5* reveals progressive defects in the inflorescence during development. Through BSA and rough mapping *tls4* has been mapped to bins 4.08-4.09 on chromosome 4 and *tls5* to bin 9.02 on chromosome 9. Fine mapping is ongoing to identify the *tls4* and *tls5* genes. We propose that these genes play a fundamental role in vegetative and reproductive development in maize.

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P208

Phase-Specific Gene Expression Patterns in Maize Leaves

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Vegetative phase change is a developmental transition in higher plants between the (often) morphologically distinct juvenile and adult phases of vegetative growth. In maize, the first 4-5 leaves are juvenile, with a waxy, glabrous epidermis, followed by 2-3 transition leaves, which have mosaic juvenile and adult morphology, with the remaining leaves being completely adult. The major effectors of the juvenile and adult phases in maize have been shown to be the micro RNAs MiR156 (Corngrass) and MiR172, but little is known about the gene networks downstream of them, which previous microarray studies (Strable et al., 2008) indicate likely involve hundreds of genes. We are examining these downstream networks using a custom microarray comprised of the maizeGDB.org high-confidence gene set and known maize microRNAs to compare the expression of juvenile, transition, and adult leaf primordia at multiple stages of development. Many more genes are found to be juvenile-upregulated than adult-upregulated and this inequality increases with increasing fold change. A majority of MiR156 loci are found to contribute to the juvenile phase while only one MiR172 locus (MiR172c) contributes to the adult phase. The absolute MiR156/Mir172 ratio is found to reach parity in leaf five. Phase-specific alterations in hormonal pathways and possible regulation by blue light are discussed.

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P209

Potato Microtubule-associated Protein SBgLR is Involved in Protein Body Formation and Its Expression Leads to Lysine Content Increase in Transgenic Maize

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Maize (*Zea mays*) seed is deficient in protein and essential amino acids,especially lysine content. Previously, we report the role of a natural lysine-rich protein gene SBgLR in increasing protein and lysine content. In this study, through seed-specific expression of SBgLR, we obtained the transgenic quality protein maize with the simultaneously increased lysine and protein contents. Using a combination of cell biology, biochemistry and molecular biology, the function of SBgLR in regulating protein accumulation during endosperm development was explored. The expression of SBgLR in maize kernels increases the accumulation of both zein and non-zein proteins. Transmission and scanning electron microscopy showed that the number of PB and proteinaceous matrix was increased obviously in T2 transgenic endosperms. Imunnogold labeling analysis showed that SBgLR was distributed toward the periphery, where microtubules (MTs) are rich, and the core of protein bodies (PBs). Our data also revealed that SBgLR promoted the expression of zein genes and lysine-rich non-zein genes. Moreover, SBgLR binds and bundles microtubules *in vitro* and *in vivo*. SBgLR may create cross-linked and/or bundled MTs in transgenic endosperm. Due to the SBgLR-MT binding, more ‘scaffold’ structures and stable intracellular environment are provided in transgenic endosperm, and it results in more PB formation and proteinaceous matrix deposition, which further increases the protein and lysine contents

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P210

Spatiotemporal regulation of environmental stress response in plants

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Throughout evolution, various strategies have evolved to enable organisms to respond to a wide array of different external stimuli. The evolution of multicellularity was also important in this process. For the first time, cells could be isolated from the external environment. This advent likely enabled a tighter control of homeostasis in some cell types, but it also required the utilization of cell-cell communication to generate coordinated responses. While these examples are intriguing possibilities, to a large extent an understanding of what principles govern environmental response in a multicellular context is theoretical. A key factor limiting progress in the field is the ability to gather biological information at cell-type or tissue-type resolution. Multicellularity has evolved independently in the plant and animal lineages, but the fundamental innovations of forming differentiated cell types and organizing these into tissues is shared. Recent technological advances have enabled genome scale and cell-type specific investigations of gene expression in the root and have placed this system at the forefront of studies on organism-environment interactions. Extensive high-resolution transcriptional maps have been generated for each cell type and under multiple environmental conditions. These data provide a clear affirmative answer to the question of whether environmental response is distinguished at the cell-type level. Still the mystery remains as to what the mechanisms are that enable these responses to be spatially differentiated. In my presentation I will discuss recent work to develop a spatiotemporal gene expression map for the salt stress response. I will also describe a new area of research we have started to understand the role of local differences in moisture during the patterning of tissues in the root in a process we term “hydropatterning”. Our work utilizes Arabidopsis, Maize and Setaria viridis as model systems.

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P211

Sterile tassel silky ear1: a new mutant with an old history

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The B-class MADS box genes, as described in the ABC model of floral development, control stamen and second whorl organ development in *Arabidopsis*, as well as in a number of divergent plant lineages. The maize mutant *sterile tassel silky ear1 (sts1)*, identified in a forward genetic screen, recapitulates some, but not all, aspects of a canonical B class mutant. We describe the cloning of *sts1*, confirming that it is a B-class gene, a homolog of *PISTILLATA* (*PI*). The unusual aspects of the mutant phenotype, relating in particular to sex determination, were investigated further and we present evidence for organ-specific abortion of the carpels in the maize tassel, regulated by *grassy tillers1* (*gt1*). In the eudicots *Arabidopsis* and *Antirrhinum*, the APETALA3- and PI-like proteins bind DNA as obligate heterodimers and regulate their own expression in an unusual autoregulatory feedback loop. In maize, a monocot, the PI homologs (STS1, ZMM18, and ZMM29) and the single AP3 homolog SILKY1 also bind DNA as obligate heterodimers. We present evidence that the STS1/SI1 obligate heterodimer goes on to regulate the expression of its own components, as well as the remaining B-class genes. Surprisingly, this obligate heterodimerization, coupled to autoregulation, has evolved convergently in the flowering plants. We have pinpointed when obligate heterodimerization evolved in the order that contains the grasses, the Poales, and identified the responsible amino acid changes. This intriguing instance of convergent evolution illuminates one aspect of the molecular complexity underlying morphological diversity.

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P212

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. If loss of katanin activity is confirmed to be the cause of the *dcd3* phenotype, our analysis has identified a new role for katanin in stomata formation in maize.

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P213

The developmental role of *ramosa1* in the evolution of grass inflorescence morphology

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The *ramosa1* (*ra1*) gene is a key regulator of maize inflorescence development and architecture, and was a target of selection during maize domestication. *ra1* encodes a C2H2 zinc-finger transcription factor with two EAR repression motifs. Maize *ra1* mutants have inflorescences with higher-order branching architectures reminiscent of some other grass inflorescences. We hypothesize that variation at *ra1* contributes to the variation in inflorescence morphology that has evolved within the grasses. Our previous studies support our hypothesis: *ra1* expression in maize, sorghum and *Miscanthus* correlates with spikelet pair meristem determinacy, and regulation of *ra1* expression timing and of RA1 activity influences the degree of branching in the inflorescence. The *ra1* gene is present in genomes of the Panicoideae subfamily that include maize and sorghum, which both belong to the Andropogoneae tribe, and taxa within the closely related Paniceae tribe, including *Setaria*. Whether *ra1* functions as a potent regulator of inflorescence architecture in other grasses is unknown. Through collaborative efforts, *ra1*, along with its native promoter, was transferred among Panicoid members maize, sorghum and *Setaria*. Initial data on cross-species expression of a *ra1* transgene reveal inflorescence architecture is impacted by transgene orthologs. These data provide a first glimpse of *ra1* function in other grasses. Our preliminary transgenic work in sorghum and *Setaria*, however, is potentially confounded by expression of the endogenous *ra1*. We are using promoter inverted repeat and designer Transcriptional Activator-Like Effector Nuclease approaches to knock out the endogenous *ra1* (*Sv-ra1*) function in the *Setaria* genome. This knockout resource will 1) provide the first insight into how *Sv-ra1* regulates inflorescence architecture; 2) create a background in which orthologous *ra1* transgenes can be introduced to study interspecific expression patterns and developmental functions. This research will elucidate cross-species function of *ra1* to broaden our understanding of how diversity in the grain-bearing inflorescence evolved.

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P214

The *fasciated ear3 (fea3)* gene encodes a receptor-related protein that regulates stem cell proliferation in maize in a pathway distinct from the known CLAVATA pathway.

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The shoot apical meristem (SAM) regulates its size during development by balancing stem cell proliferation and the incorporation of daughter cells into primordia. Several "fasciated" mutants with enlarged meristems have been identified in maize, and can be used to study the genetic basis of meristem size regulation. In previous work, we isolated two maize genes, *thick tassel dwarf1* (*td1*) and *fasciated ear2* (*fea2*), which are homologous to the Arabidopsis leucine-rich-repeat (LRR) receptor- genes CLAVATA1 (CLV1) and CLV2, respectively. CLV1 and CLV2 are activated by the CLV3 ligand and repress the stem cell promoting transcription factor WUSCHEL.

Here we present a phenotypic and molecular characterization of the maize mutant fea3 that causes the over-proliferation of the inflorescence meristem, leading to enlarged or fasciated meristems. We cloned *fea3* using a map-based cloning approach and the mutant results from an insertion of a partial retrotransposon into an exon of *fea3*. We confirmed this identity by isolation of three additional alleles from a targeted EMS mutagenesis. *fea3* encodes a predicted leucine rich repeat receptor-like protein, related to *fea2*. In-situ hybridization and RFP-tagged transgenic plants show that FEA3 is expressed in organizing center of SAM and is also expressed in the root apical meristem. FEA3 is localized in the plasma membrane. To ask if FEA3 responds to a CLV3- related (CLE) peptide, we tested its sensitivity to different peptides. *fea3* mutants showed reduced peptide sensitivity, but interestingly they responded to a different CLE peptide compared to FEA2. Double mutants of *fea2/fea3* and *td1/fea3* have additive and synergistic fasciated phenotypes, indicating that they act in independent pathways that converge on the same downstream target to control meristem size. These results indicate that the function of FEA3 as a receptor protein is in a new pathway distinct from that of TD1 and FEA2.

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P215

The maize Gα-subunit COMPACT PLANT2 interacts with a CLAVATA LRR receptor-like protein to control shoot meristem size

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Plant growth and development depend upon the balancing of meristem proliferation that is controlled by a negative feedback loop between the *CLAVATA* pathway and the *WUSCHEL* homeobox gene. CLAVATA signaling involves a secreted peptide, CLAVATA3 (CLV3), and its perception by cell surface leucine-rich repeat (LRR) receptors, including the CLV1 receptor kinase, and a LRR receptor-like protein, CLV2. However, the signaling mechanisms operating downstream of these receptors are not fully understood, especially for the LRR receptor-like proteins, which lack an intracellular signaling domain. Here we show 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 by interacting with the maize LRR receptor-like protein FASCIATED EAR 2 (FEA2) to control the shoot meristem size. The phenotypic analysis suggests that *ct2* mutants exhibit meristem proliferation defects similar to *CLAVATA* mutants, such as maize *fea2*. Genetic data indicate that *ct2* is epistatic to *fea2* with respect to spikelet density, which correlates with meristem size, and suggests they act in a common pathway. The co-immunoprecipitation data further suggest that FEA2 specifically interacts with CT2 *in vivo*. To gain a better understanding of the crosstalk between G protein signaling and the CLAVATA pathway, we are using Fluorescence Resonance Energy Transfer (FRET) and other biophysical methods to investigate the factors that affect signal transmission between FEA2 and CT2. This research introduces a new paradigm in G protein signaling, because G proteins interact exclusively with 7 pass transmembrane receptors in mammals and fungi.

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P216

The maize nucellus contributes to early kernel development through cell cycle arrest accompanied by post-pollination expansion

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The nucellus is the central tissue of maize ovules that gives rise to and supports the female gametophyte. Although this tissue constitutes a large part of the ovule for many species, including maize, relatively little is known about its development or pattern of gene expression. During experiments with post-pollination maize ovules, we observed expansion of the nucellus concomitant with arrest of its cells in G2 of the cell cycle. Comparison of RNA-seq data from mature, unpollinated and 6 day post-pollination maize ovules confirmed nucellar G2 arrest by the complete absence of B cyclins in the post-pollination sample. Arrest of nucellar cells in G2 coincided with increased expression of genes encoding cell wall weakening enzymes. This included nucellus-specific expansins, which accounted for a large proportion of sequence reads from both libraries. One expansin in particular, resulting from a recent gene duplication, is expressed at a very high level. The RNA-seq data supports the hypothesis that the maize nucellus expands rapidly following fertilization, and creates fragile cells that facilitate endosperm and embryo enlargement. The RNA-seq data reveals physiological mechanisms that highlight the role of nucellus in early kernel development.

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P217

The Mechanism of Moisture Sensing in Plant Roots

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Water is necessary to all known forms of life, and performs many diverse roles within plants. The root system takes up water and nutrients from the soil, and it must dynamically alter its growth and development in response to external stimuli to optimize these functions. It is currently unknown how roots perceive water in the environment, or how this signal is transduced to lead to developmental changes. Our group has observed that many aspects of root development can be patterned in a manner that correlates with the distribution of water around the root. We have coined the term "hydropatterning" to describe this developmental response, and aim to uncover the mechanism of moisture sensing in plant roots by studying this process in Arabidopsis and maize. This combines the molecular genetic toolkit of Arabidopsis with manual dissection and micromanipulation techniques made possible with the large roots of maize. A proof-of-concept experiment using dissected maize roots has shown that regions of the root that develop while contacting a wet surface are transcriptionally distinct from regions exposed to air. In addition, a method of rapidly phenotyping maize roots for changes in the hydropatterning response has been developed. This has been used to identify variation in the process within the nested association mapping (NAM) population, and is currently being used for a forward genetic screen. Future work will involve determining the nature of the environmental stimulus perceived by the roots, identifying which regions of the root are sensitive to this stimulus, and characterizing molecular changes downstream of its perception. In addition, the genes involved in both perception and transduction of this signal will be identified and characterized. Results will further understanding of how plants sense water in the environment, and how this provides a signal to alter development of the root system.

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P218

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

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Axillary meristems (AMs) are small groups of stem cells produced in the axils of leaf primordia. AMs generate shoot branches (eg., maize tillers) and inflorescences (eg., maize tassels and ears), and thus are important in determining the architecture of higher plants. Previous studies revealed several critical genes functioning in auxin biosynthesis, transport or signaling to regulate AM production. We have identified a new mutant *barren stalk2* (*ba2*) which produces no ears, maps to chromosome 2.04 and is likely the lost mutant first described by Hofmeyer in 1930. The *ba2* mutants have fewer tassel branches and spikelets and a higher percentage of single spikelets than the normal plants. Scanning Electron Microscope analysis shows that the *ba2* mutants have defects in reproductive AM formation. The *ba2* mutation suppresses tiller growth in the *teosinte branched1* mutant, suggesting an essential role in vegetative AM formation. The *ba2* gene was positionally cloned. The *ba2-3112* and *ba2-EV* mutant alleles have a nonsense mutation at the 3' or 5' coding region, respectively, and the *ba2-DS* mutant allele has a *DS* transposon insertion ~0.2 kb 5' of the start codon. Phylogenetic analysis shows that the *ba2* gene encodes a protein with a plant-specific conserved domain involved in protein-protein interaction. Characterization of the genetic interaction between *ba2* and other genes functioning in AM production demonstrates that the *barren stalk1* (*ba1*) gene, which encodes a basic helix-loop-helix transcription factor essential for AM formation, is epistatic to *ba2* and shows a dosage effect in *ba2* mutants. Additionally, BiFC (bimolecular fluorescence complementation) assays show that the BA2 and BA1 proteins co-localize and interact in the nucleus of tobacco. These data suggest that *ba2* and *ba1* act together in the same pathway for AM production.

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P219

The Polycomb Group Gene *EMF2B* regulates floral meristem determinacy in rice

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The floral meristem (FM) is determinate, i.e. terminates after the production of a finite number of floral organs. In Arabidopsis FM determinacy is established by MADS-box transcription factors encoded by the *AGAMOUS* (*AG*) and *SEPELLATA* (*SEP*) genes, and in maize by the *Bearded Ear* locus (orthologous to Arabidopsis *AGL6*) and the *ZAG* loci (orthologs of *AG*). Here we show that FM determinacy in rice is additionally under the control of a Polycomb Group (PcG) gene involved in chromatin silencing. Polycomb Group proteins act in a chromatin-remodeling complex called the Polycomb Repressive Complex 2 (PRC2) that represses the transcriptional activity of target genes. In Arabidopsis, the PRC2 does not play an essential role in floral meristem (FM) determinacy but functions in a secondary pathway where the PRC2 is recruited by *AGAMOUS* to silence *WUSCHEL* thus terminating the FM. We characterized mutants in the rice *EMF2B* gene, an ortholog of the Arabidopsis PRC2 gene *EMBRYONIC FLOWER2* (*EMF2*). Loss of *EMF2B* in rice results in stunted plants that are completely sterile. *emf2b* mutant flowers have severe floral organ defects and meristem indeterminacy. Transcriptome analysis identified a number of E-function genes (*SEP* and *AGL6-like*) that are differentially expressed in the *emf2b* mutant compared to wild type. Loss-of-function mutants of several of these genes in rice and maize resemble *emf2b*. Chromatin immunoprecipitation was used to identify genes that are candidates for direct repression by EMF2B. We propose that the PRC2 plays a major role in floral meristem determinacy in rice through the regulation of E-function floral organ specification genes. Our results suggest that rice has evolved a distinct mechanism from Arabidopsis in the utilization of chromatin silencing by the PRC2 complex to achieve the determinate state of the FM, a mechanism that might be applicable to other grasses such as maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P220

The role of TANGLED in division plane orientation

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The plant body is shaped by three fundamental cellular processes: division, expansion and differentiation. 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. Moreover, division plane orientation is important for all eukaryotic cells, and plants serve as useful model organisms for studying this process. Little is known about the factors that control proper orientation of the division plane in plants, however, recent data suggests that sequential modification of the plant division site is essential for the final proper orientation of cell division. This research identifies the proteins responsible for controlling division plane orientation in both maize and *Arabidopsis thaliana* using TANGLED, a division site marker, as bait in yeast two hybrid and immunoprecipitation followed by mass spectrometry. Many of these proteins have potential roles in membrane reorganization and localize to the growing edges of the cell plate, suggesting that TAN may coordinate essential membrane reorganization as the cell plate reaches the cortex. Finally, live cell imaging in maize is used to explore the function of TANGLED and other division plane proteins during the cell cycle.

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P221

The *Suppressor of sessile spikelet* loci regulate the production of paired spikelets in maize

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The *Suppressor of sessile spikelet* mutants are semi-dominant mutants characterized by the production of single instead of paired spikelets, leading to gaps between the rows on the ear and a sparse tassel. *Sos1* has been fine mapped to a single BAC in bin 4.02 containing three predicted genes. *Sos2* has been mapped to a region of chromosome 10.01 containing ~40 predicted genes, and *Sos3* has been mapped to chromosome 1 between bins 1.06-1.07. Therefore, at least three loci regulate the production of the paired spikelet in maize. Each of the mutants also has defects consistent with additional roles in inflorescence development. *Sos1* has a smaller apical inflorescence meristem resulting in a further reduction in row number. *Sos2* frequently has an aborted apical inflorescence meristem indicating a function in meristem maintenance. Severe *Sos3* mutants have unbranched tassels or tassels with a few short branches. As the production of paired spikelets is a derived trait found in all 1000 species in the Andropogoneae, but absent from more distantly related grasses including rice, barley and wheat, an understanding of the role of the *Sos* genes will shed light into the development and evolution of a novel inflorescence character.

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P222

Towards the positional cloning of *Few-branched1*, a bract suppression mutant in maize

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Phytomers, the basic structural units of plant development, consist of a leaf, an axillary meristem, and an internode. Differential allocation of growth among these components results in vast structural diversity across species. The grass family, Poaceae, has evolved complete leaf suppression in the inflorescence, correlated with a highly-branched tassel as in *Zea mays*. Inflorescence leaves are also called bracts, and when their suppression is disrupted, bract growth occurs at the expense of tassel branches. A number of related bract suppression mutants in maize have recently been isolated, enabling assembly of the bract suppression gene network and therefore an improved understanding of morphogenesis. In addition, bract suppression mutants afford an opportunity to investigate convergent evolution, as Brassicaceae—the family to which *Arabidopsis* belongs—independently evolved bract suppression. Mechanisms and gene functions in bract suppression can be easily compared between maize and *Arabidopsis*.

The semi-dominant *Few-branched1* (*Fbr1*) mutant exhibits reduced tassel branching and ectopic bract outgrowth in the inflorescence. Though *Fbr1* was originally isolated by M.G. Neuffer in 1987, it remains unmapped. We are currently mapping *Fbr1* using a marker-based, positional cloning approach and have localized it to a 1.2 Mbp region containing roughly 50 candidate genes. Additionally, we are characterizing early developmental stages of mutant tassel inflorescences using scanning electron microscopy to follow early stages of bract growth and determine if branches fail to initiate, or initiate but then abort.

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P223

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 forms the outermost layer of the endosperm, is the primary contributor of important dietary benefits of cereal bran and is also the major source of hydrolases paramount for the malting industry. The research described here seeks to explore the gene regulatory networks (GRN) controlling maize endosperm cell differentiation and development via analysis of an aleurone development mutant, naked endosperm (nkd). Nkd endosperm is characterized by defects in aleurone cell fate and differentiation traits, including sporadic expression of a transgene aleurone identity marker, Vp1pro:GUS. The nkd mutant is conferred by duplicate genes encoding Indeterminate (ID) domain zinc finger transcription factors. The genetic identities of nkds were verified by independent nkd1-Ds and nkd2-Ds alleles which failed to complement the original nkd mutant. The lack of transcripts detectable by RT-PCR suggests that one nkd1-Ds and two independent nkd2-Ds alleles are nulls. Current efforts seek to define the genes regulated by the NKD transcription factors. To precisely monitor the downstream genes and developmental processes regulated by NKDs, a transcriptomic study involving the RNA-seq analysis of WT and Nkd mutant is being undertaken using endosperm cells captured by Laser Capture Microdissection (LCM). Furthermore, a SAAB assay is currently being pursued to characterize the NKD DNA-binding properties and consensus sequence. The binding of NKDs to the Vp1 promoter was tested and confirmed with GMSA. A transient assay is currently ongoing to determine if NKD can induce Vp1 expression in vivo. Upon identification of the binding consensus sequences, we will search for this motif in the promoter regions of differentially regulated genes identified in the RNA-seq experiment to shed light on the direct and indirect downstream GRN controlled by NKDs.

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P224

Transcriptional repression mediated by REL2 and REL2-LIKE co-repressors in the development of maize inflorescences

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Transcriptional repression is emerging as a crucial mechanism for the regulation of different plant developmental pathways. It can be divided into two types, active and passive repression. Passive repression generally occurs when a protein binds to a transcriptional activator and prevents transcription by steric hindrance. In contrast, active repression includes the process by which proteins interact directly with DNA and with other non-DNA-binding proteins such as co-repressors to block transcription. An increasing number of plant developmental pathways are found to be negatively regulated by co-repressors belonging to the Arabidopsis TOPLESS (TPL) family. TPL lacks intrinsic DNA binding ability but is recruited by different transcription factors containing plant-specific EAR repression motifs.
We previously characterized a maize functional homolog of TOPLESS, the *(ramosa1 enhancer locus2 (rel2). rel2* mutants were originally identified in a forward genetic screen and show distinct phenotype in both male and female maize inflorescences. REL2 was shown to interact with the EAR-motif containing transcription factor RAMOSA1 (RA1) to prevent the formation of long branches in both ear and tassel. In maize there are three closely related but so far uncharacterized members of the REL2 family, REL2-LIKE1, REL2-LIKE2 and REL2-LIKE3. We have cloned the three genes and identified insertional mutants to characterize their function during development. We are using these lines to create double and triple mutant combinations to analyze the degree of functional redundancy existing in this family of co-repressors. We will present preliminary protein-protein interaction data of all REL2/REL2-LIKE proteins with known EAR-containing transcription factors regulating inflorescence development in maize. Our goal is to characterize the molecular mechanisms regulating the development of maize inflorescences and the contribution of transcriptional repression in the various pathways that give rise to complex reproductive structures.

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P225

Wab1 encodes a TCP transcription factor and regulates LG1 expression

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The maize leaf is composed of two major tissues, a distal blade that tilts away from the stem and the more proximal sheath that tightly wraps around the stem. At the junction of blade and sheath, the ligule and auricles are found. The auricles act as a hinge to let the blade lean back and the ligule is a flap of tissue, preventing water from entering into the stem. Our goal is to understand how cells in a leaf primordium differentiate according to position and adopt specific cell types. We are using a number of maize mutants that affect patterning in the leaf. liguleless1 and liguleless2 remove the ligule and auricle, while Wavy auricle in blade (Wab1) has ectopic auricle in the blade. Positional cloning identified two genes that may correspond to the Wab1 locus. One of which, a TCP transcription factor related to TEOSINTE BRANCHED1, is ectopically expressed in Wab1 leaves. We identified a revertant and found a mutation in the conserved TCP domain. The revertant has upright tassel branches similar to a mutant in the same TCP gene that was identified by its tassel phenotype, branched angle defective (Bai et al., 2012). We developed an antibody to LG1 to determine how the protein localizes at the region of the ligule and in the tassel. In wild-type leaves, LG1 is expressed in a narrow band starting at leaf 6 just before formation of the preligule band. Expression is mostly epidermal, but internal at leaf 7 near vascular structures. Not all small cells in the preligule band express LG1. Expression is earlier and broader in Wab1 mutants, with expression extending up into the blade. LG1 is also expressed at the base of tassel branches, fitting with the loss of function Wab1 phenotype.

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P226

Discovery of sequence unique to Abnormal Chromosome 10 in *Zea mays* yields a novel kinesin expressed in meiosis

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Abnormal chromosome 10 (Ab10) is a rare chromosome variant of normal chromosome 10 found within the genus Zea. The haplotype was first identified by Marcus Rhoades in 1942. Ab10 is distinguished from N10 by an extrachromosomal region containing "knobs": heterochromatic regions composed of either a 350-bp or 180-bp repeat motif. These same knobs are located throughout the maize genome and, in the presence of Ab10, form neocentromeres and segregate into upwards of 70% of the progeny during meiosis instead of the expected Mendelian 50%. This process of preferential segregation is referred to as meiotic drive.

The genes that cause neocentromere movement and meiotic drive are unknown. The way knobs move laterally along spindle fibers suggests they attach to a microtubule motor protein, such as a kinesin. Here we report the discovery of the first novel Ab10 sequence as well as Kin618, a C-terminal kinesin unique to the Ab10 haplotype. Kin618 is not expressed in an Ab10 mutant line deficient for meiotic drive, making it a strong candidate for the gene that causes neocentromere movement and meiotic drive.

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P227

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

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Some fast flowering lines of maize have been produced in the past but all have had several drawbacks for experimental purposes. We report here the development of a fast flowering mini-maize with robust characteristics that should expedite maize studies or that could be used as an educational tool. Mini-Maize can produce five generations per year easily, but six generations could likely be achieved if conditions are optimized. Mini-Maize is about 30 cm tall when mature, and can be grown closer together and in smaller pots than normal maize. Mini Maize is a descendent of a double-cross hybrid of Neuffer's Early ACR line by Alexander's Early Early Synthetic, and Tom Thumb Popcorn by Gaspe Flint. Offspring were selected through alternating back-crosses to Tom Thumb Popcorn and Gaspe Flint. Selection was based on fast flowering and desirable plant morphology including high pollen yield, high seed count, ear shape, rapid ear maturity and a husk that protrudes from the leaf sheath before silking. Selected individuals were used to initiate a selfing regime for 11 generations with continued selection for desired traits. Flowering is between 28 and 35 days after planting depending on environmental conditions, and seeds can be harvested at day 60; thus introgressions to this line can be accomplished quickly. Pollen mother cells can be found in the pachytene stage approximately 24 days after planting. Introgressions of the simple genetic markers *y1* and *R-scm2* are currently in progress. A FISH karyotype has been generated, and sequencing of this line is in progress. Fast-Flowering Mini-Maize can serve as a short generation model system within maize itself.

P228

Functional centromere lost centromeric specific sequences but gain new sequences from nearby chromosomal arm

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The centromere is the constricted region of a chromosome that serves as the assembly site of the kinetochore and participates in the regulation of chromosome movement during mitosis and meiosis. Epigenetic modifications are essential for the formation and proper functions of centromeres. Here we identified a newly formed dicentric chromosome in maize from intrachromosomal recombination and BFB cycles, in which only one centromere is active. The centromeres lost CentC sequences and dramatic reduced the CRM sequences, but when the molecular features of functional centromeres such as CENH3 was examined, they were present. Immunolocalization analysis of phosphorylation of H3T3, H3ser-10 and H2A levels on this new centromere shows a pattern typical of a functional centromere. Meiotic analysis revealed that this dicentric chromosome is stable and transmit very well. To examine the new sequences associated with CENH3 in this centromere, chromatin immunoprecipitation (ChIP) was carried out with anti-CENH3 antibodies and material from young seedlings with or without dicentric chromosome. We mapped the ChIP-Seq reads to the reference genome and found a 723kb region from the short arm of maize chromosome 9 involve the new centromere formation. This region is gene-poor and full of TEs, but genes in this region are transcribed. The original 723kb region shows a high DNA methylation level as native centromeres but had no significant change when it involved into new centromere formation. The reactivation of this newly formed centromere indicated that centromere reactivation may not dependent on the relatively intact DNA sequences or topology of original inactive centromere.

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P229

Maize Whole Chromosome Exon Paints Applied to Related Species

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Whole chromosome exonic paints have been developed for maize chromosomes 1, 5, and 8. They consist of a collection of bioinformatically designed probes corresponding to the exons predicted from the genomic sequence. When used as a probe onto somatic maize chromosomes, they label the respective pair. Using different fluorophores for different chromosomes allows one to distinguish translocations between the respective chromosomes and potentially other single cell chromosomal aberrations. Because the probes are composed of genic sequences, they were assumed to hybridize to relatives of maize. We applied the paints for chromosomes 1 and 5 to *Tripsacum dactyloides*, *Sorghum bicolor* and Saccharum to visualize the changes in chromosomal organization relative to maize. Chromosome 1, but not chromosome 5, is relatively intact among maize, Tripsacum, and sorghum. Analysis of the Saccharum hybridization pattern is in progress. Cytogenetically derived results take relatively little time to obtain and can be utilized to corroborate molecular data or provide novel insights into the chromosomal evolution of related taxa.

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P230

PPR-Protein Sequences Hybridized to Maize Chromosomes

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Genes that encode pentatricopeptide repeat (PPR) proteins are abundant in plants. Over 450 PPR proteins are encoded in the B73 maize nuclear genome and nearly all of them have been found to have organellar targeting sequences. PPR proteins are thought to be involved in organellar RNA editing, processing, and translation. The purpose of this experiment was to use fluorescence in situ hybridization (FISH) to test how well gene-specific PPR probes could be visualized on chromosomes. Fifteen PPR-protein coding sequences were found within a 1.3 Mb region on the long arm of chromosome 2. Two of these coding sequences, referred to as gene 5 and gene 6, were tested for their ability to specifically hybridize to chromosome 2. These genes have mitochondrial targeting sequences and share regions of high similarity. A BLAST search showed hits for parts of these genes on various chromosomes; however, only one hit (on chromosome 1) was long enough to potentially show detectable signal by FISH with the probes for our genes 5 and 6. Each of the probes hybridized strongly to the long arm of chromosome 2 in B73 root tip metaphase spreads. Both probes also hybridized, but to a much lesser extent, to the end of the long arm of chromosome 1. These findings are in line with expectations from the B73 sequence data. To our knowledge, this experiment is the first demonstration of the utility of FISH to confirm the locations of PPR-protein coding sequences on chromosomes.

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P231

The collection of maize meiotic mutants illuminates the process of initiation of recombination during the leptotene stage

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The initiation of recombination during meiosis is a critical step that regulates the position of hotspots and crossovers. Meiotic recombination is initiated by the introduction of programmed DNA double strand breaks (DSBs) formed during leptotene by the DNA transesterase SPO11. While the SPO11 protein is well conserved its partners have evolved more rapidly. Maize contains three genes coding for SPO11 as in Arabidopsis. We want to discover and analyze the SPO11 complex in maize. We are taking advantage of the large maize meiotic mutant collection. We have cloned two allelic maize mutations, mtm99-14 and mtm00-03. Both alleles completely delete the SPO11-1 gene. We have characterized two more spo11-1 insertion alleles by reverse genetics. All the maize spo11-1 alleles show meiotic defects that mainly lead to asynapsis and univalent formation. Most of spo11-1 mutants meiocytes show complete absence of DSBs by TUNEL assay and absence of RAD51 foci in the mutant nuclei. However by contrast to what is described in spo11 mutants in other species, around 6% of meiocytes analyzed show residual signs of recombination leading to one to two bivalents. This data suggests a minor SPO11-1 independent DSB formation pathway in maize. In addition to these early recombination defects, cytogenetical analyses show other chromosomal meiotic abnormalities in spo11-1 mutants. These data show a link between the initiation of recombination and axial element conformation predicted but never observed in other species. In order to investigate if this link can be observed in other early recombination maize meiotic mutants, we identified the maize homologs of the known Arabidopsis DSB factors (PRD1, PRD2, PRD3, DFO). We have identify a small deletion in a known meiotic gene in asynaptic (as1), the first maize meiotic mutant discovered [Beadle and McClintock 1928 Science]. Preliminary data suggest that AS1 is also involved in recombination initiation.

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P232

ZIP1 and SMC6-independent centromere association for pairing initial in maize

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In most eukaryote species, segregation of homologous chromosomes during meiosis is established through homologous chromosome pairing, synapsis, and recombination. The underlying mechanism of the initialization of homologous chromosome pairing is poorly understood. We found that functional centromere association begins at the leptotene stage and is earlier than the formation of the telomere bouquet. The centromeric specific sequences are not involved in the homologous pairing initial process which is dependent on the centromere activity. Maize ZYP1 is not beginning to deposit on centromere regions at leptotene, but it is loaded onto the chromatin at multi-regions when SC forming. Two Structural Maintenance of Chromosomes (SMC5 and 6) elements were cloned and well characterized . Immunostaining results showed that ZmSMC6 do not take part in the initialization of chromosome pairing, but is a novel central element of synaptonemal complex. Results from meiotic mutant afd1 and ZmSMC6 RNAi lines indicated that centromere association and CENH3 may play an important role in homologous pairing initialization.

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P233

Chromosome painting in an undergraduate genetics laboratory

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Chromosome painting is an important molecular genetic technique with a wide variety of applications in science and medicine. Images of fluorescently-labeled chromosomes are often highlighted in undergraduate textbooks because they are visually stunning and they have “seeing-is-believing” educational value. Despite the fact that chromosome painting utilizes many standard genetic techniques typically taught in undergraduate laboratories, most educators overlook chromosome painting as an exciting laboratory exercise. During a sophomore-level introductory Genetics course, I introduced students to fluorescence *in situ* hybridization (FISH) on maize chromosomes. Throughout this semester-long laboratory experience 18 students met once a week for three hours and completed activities including: plasmid DNA isolation, PCR, fluorescently labeling DNA, producing slides from maize root tips, hybridization of probe and slide, and finally capturing images from the hybridized slides using a confocal microscope. While all of these methods will be described, I will provide detailed troubleshooting tips on slide preparation and hybridization conditions. Upon reflecting on this experience, students appreciated learning genetic techniques during a semester-long goal-oriented investigation. FISH can be readily implemented in an undergraduate course as an exciting introduction to genetics techniques.

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P234

Maize Outreach Program Targeting Title I Middle School Students with the Involvement of Community Members

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We have developed a unique and multi-pronged outreach program involving teachers and gardeners to educate and engage middle school students from Title I schools as well as other youths. Science outreach activities are being designed to teach students about grains, including maize, as well as various aspects of plant biology, nutrition, and sustainable agriculture. We are creating activities to reach students both inside and outside the classroom. Laboratory demonstrations and exercises are being implemented to expose students to science research within their classrooms and to explore the nutritional properties of maize. Additionally, we have students visit the college campus to tour laboratory facilities and the greenhouse where they can participate in maize cultivation. In cooperation with a local community garden, a seasonal grain garden has been established, containing a variety of grains (including maize) in rotation with cover crops. The location of the garden makes it highly accessible to families of different backgrounds, groups serving disadvantaged youth, local volunteer clubs, and Title I elementary and middle school students. Children are involved in the tilling, planting, and maintenance of the garden and are taught basic plant morphology and physiology. Once the plants are fully grown, they will serve as teaching tools to help students identify the different grains and learn about their uses. This project was funded by the NSF (IOS-0923880).

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P235

Online Guide to Maize Mutant Phenotypes

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Our Online Guide to Maize Mutant Phenotypes is now available. We present many mutant maize phenotypes that have been photographed and described by colleagues and myself (MGN) in my 65 years as a maize geneticist. Currently, 806 high-quality images have been selected to describe, as completely as possible, the diversity of expression in maize. The images are arranged into broad categories (morphology, stature, color, etc.) using established maize phenotypes (dwarf, lesion, ramosa, knotted, etc.). Each phenotype has a brief definition and one good representative image, or in some cases (e.g. virescent) multiple images demonstrating the range of expression of that phenotype. Captions describe each picture in more detail, and in some cases include information such as effects of background, temperature, etc. The Guide is intended for a broad audience, from someone visiting a geneticist's corn field for the first time to established researchers who might want to compare an interesting plant variation with those previously described. We invite your suggestions and additional images and materials to help improve and expand this resource.

The Informatics Research Core Facility at the University of Missouri (Scott Givan, Associate Director) is providing informatics and programming support for my own "Mutant Database" and for generating the web pages for our *Online Guide* from it.

We are grateful to MaizeGDB for hosting the Guide and for the generous donation of their time and services. Support from NSF grant *IOS-1239861* for current work is gratefully acknowledged. I would also like to thank the NSF, USDA, and Pioneer Hi-Bred for their support throughout the years.

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P236

Panzea: Education and outreach for the Maize Diversity project

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The Maize Diversity Project (NSF # 0820619) is a multi-institutional collaborative project investigating the genetic architecture of complex traits in maize and teosinte (http://www.panzea.org). A number of exciting educational items have been generated by the project, including a traveling science museum exhibit, associated teacher materials, and regular workshops on the latest Genotyping by Sequencing (GBS) technologies.

The science museum exhibit "Maize: Mysteries of an Ancient Grain," focusing on evolution and genetic diversity through the study of maize, has been viewed by over 300,000 visitors so far and is on target to reach one million viewers during its travels, which have included the Corn Palace (Mitchell, SD) as well as museums and libraries. Contact information and more about the exhibit is available at http://maizeexhibit.org/. Associated with the exhibit is the online Teacher Friendly Guide™ to the Evolution of Maize, available (http://maize.teacherfriendlyguide.org) and also as hard copies. In addition, the project is collaborating with Cornell's Computational Biology Support Unit (CBSU) to host GBS workshops every three months. The workshops are two days long, team taught, and include both lectures and hands-on data analysis exercises. Nearly 250 people have attended these workshops to date, coming from all over the world, and there is a continual waitlist. Videos are available online, as well as CDs.

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

P237

Structure and transcription of maize genes that exhibit B73/Mo17 presence -absence variation

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Array-based comparative genomic hybridization (CGH) experiments (Springer et al. 2009) have revealed levels of genic presence-absence variation (PAVs) that are unprecedented among higher eukaryotes. PAV identified by CGH does not reveal the length and location of the absence, or the impact on transcription, relative to an intact gene. By studying a small number of genes classified by Springer et al. (2009) as exhibiting PAV, we hope to identify structural and transcriptional commonalities which will allow for a deeper understanding of the nature of the absence and its variation among maize haplotypes. To elucidate the prevalence PAV beyond B73 and Mo17, PCR was performed across the length of several genes for seven haplotypes. In addition, we have used RNA-Seq analyses from several developmental stages to characterize the level of transcription in PAV genes from B73 and Mo17. This study was largely conducted during investigative laboratory experiences in introductory and advanced undergraduate genetics courses.

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P238

Test cros performance and combining ability of selected QPM lines

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One hundred thirty four genotypes were evaluated by simple lattice design during 2007 cropping season at Awassa and Melkassa, Ethiopia. In this study a released QPM check BHQ542 with other checks were used at both locations. The objective of the study were to determine performance of the top crosses, combing ability and heterotic patterns of the 44-QPM inbred lines and testers, to estimate the correlation among the traits measured and to identify some promising crosses for future advanced trial. All agronomic data were measured and analyzed Using SAS software. Significant differences among genotypes were recorded for most of the traits except for number of ears per plant, disease and lodging. Similarly, there was significant difference among crosses for most of the traits at both sites except for days to emergence, ear height, ear diameter, number of seed per plant, and 1000 seed weight. Sixteen crosses at Awassa and four crosses at Melkassa were superior to BHQ542 in yield performance but no crosses out yielded this check in the combined data. However, five lines crossed with tester(T1) and seven lines crossed with tester2(T2) gave superior yield than the second best common check BH540 in the combined data. The crosses that out yielded BHQ542 and BH540 at individual location and over locations could be utilized for future maize improvement activities. Heterosis was varied highly and the degree of heterosis manifestation for grain yield among crosses was higher at Awassa and lower at Melkassa. The GCA due to testers was highly significant for few traits at Awassa but highly significant for most of the traits at Melkassa. The SCA mean square was significant few traits at both locations. Thus, for most of traits studied additive genetic variance was more important than the non-additive genetic variance in controlling the inheritance of traits.

Funding acknowledgement: NORAD and CIMMYT

P239

The Maize Germplasm Bank at CIMMYT : An Invaluable Genetic Resource for Maize Geneticists, Breeders, Producers and Consumers throughout the World

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The Maize Germplasm Bank (MGB) at CIMMYT (The International Maize and Wheat Improvement Center) in Texcoco, Mexico, holds the world's largest collection of maize germplasm, currently numbering over 27,000 seed accessions, in a subterranean, earthquake-proof, temperature- and humidity-controlled vault. Although the CIMMYT has been distributing seed of landraces, improved materials and CIMMYT inbred lines (CMLs) to researchers, breeders and farmers for nearly 50 years, only recently has the seed request process been available to the public via the CIMMYT website (www.cimmyt.org/obtainseed). In addition, the MGB database will be uploaded to the new GRIN-Global system in 2013, and for the first time will be publically accessible online. In order to achieve and maintain the highest quality seed for our global clientele, the CIMMYT Maize and Wheat Germplasm Banks jointly achieved ISO9001:2008 certification in December 2012. We are only the third germplasm bank in the world (and the first outside of Europe) to achieve certification. In addition to the maize collection, the MGB holds over 200 seed accessions of the closest wild relatives of maize, the "teosintes" (Zea mays sensu lato and other Zea species), as well as a live germplasm collection of the perennial sister genus to Zea, the genus Tripsacum. The MGB has been providing landrace germplasm for the GEM (Genetic Enhancement of Maize) Project and for ongoing genotypic and phenotypic evaluation by the CIMMYT-MasAgro "Seeds of Discovery" (SeeD) Project, and is currently conducting targeted phenotypic surveys of the germplasm collection, also in collaboration with SeeD. All of these efforts are part of the MGB's mandate to preserve in perpetuity the genetic diversity of maize and to facilitate the use of these genetic resources to meet the challenges of achieving food security for the world's growing population.

P240

A genome-wide association study of a naturally-occurring flecking phenotype identifies genes associated with disease resistance.

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A naturally occurring 'flecking' phenotype in maize, usually observed as mild chlorotic spots, is often considered a marker for broad-spectrum disease resistance. The genetic basis of this phenotype is completely unknown. In a diversity panel of 279 lines, the mild lesion mimic phenotype was shown to be significantly positively correlated with increased disease resistance to southern leaf blight, northern leaf blight and grey leaf spot and with a spontaneous HR phenotype induced by an auto-active resistant gene. A genome wide association analysis was performed in this population to identify loci associated with variation in flecking. The analysis was based on 263,145 SNPs, using a linear mixed model that controlled for spurious associations due to population structure. We identified several candidates, including genes involved in vesicle trafficking and defense-related multidrug efflux, a proteinase inhibitor, cytochrome P450, cytochrome c oxidase assembly protein PET191 and a transmembrane amino acid transporter family gene. Mild flecking was shown to be associated with increased levels of reactive oxygen species, suggesting a link with the hypersensitive defense response.

Funding acknowledgement: United States Department of Agriculture (USDA)

P241

A maize domestication QTL for internode length in the ear maps to a YABBY transcription factor that controls shattering in *Sorghum*.

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The maize gene *teosinte branched1* (*tb1*) is a 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. These additional QTL interact epistatically with *tb1*, and thereby enhance the effects of *tb1*. One of these additional QTL is called *enhancer of tb1.2* (*etb1.2*) and it affects internode length in the ear and inflorescence sex (staminate or pistillate). We fine-mapped *etb1.2* to an ~68 kb region on the long arm of chromosome 1. This 68 kb region contains exon 1 of a YABBY gene as well as ~67.8 kb of 5' upstream sequence. This YABBY gene is similar to *YAB2* gene in *Arabidopsis*. Since there are no amino acid differences between maize and teosinte for exon 1, a *cis* regulatory change is likely the causal polymorphism. Expression assays indicate that the teosinte allele is expressed 10-fold higher than the maize allele. Interestingly, this specific YABBY gene was recently shown by Lin *et al.* (2012) to control the loss of seed shattering during sorghum domestication. Lin *et al.* also suggest that this gene controlled the loss of shattering during maize domestication. Thus, *etb1.2* appears to act as key regulatory locus for multiple domestication traits including internode length, inflorescence sex and shattering.

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P242

A Parallel Selection Experiment Aimed at Elucidating the Genetic Architecture of Tropical to Temperate Adaptation.

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The genetic diversity within U.S. farmed maize represents only a fraction of the diversity of maize globally. The lack of genetic diversity within U.S. maize may constrain the potential for trait improvement and it raises concerns about the vulnerability of the U.S. maize crop to biological and environmental stresses. Using other germplasm with unique, putatively useful variation would help to mitigate these issues, and a rich source of diversity is available from tropically adapted maize germplasm. However, adapting tropical material to temperate climates can be highly inefficient due to major genetic barriers including photoperiod sensitivity. To overcome this barrier it is necessary to further our understanding of the genetic architecture underlying the response to artificial selection across multiple environments. For this purpose, a randomly intermated synthetic population was developed from seven tropical inbred lines. Using a standardized selection protocol for early flowering, a parallel selection experiment is being conducted across a latitudinal transect of trial sites from WI to PR. High-density genotyping is being used to examine the within and across generational QTL associated with phenotypic variation and population improvement in flowering time. The latest results of this study will be presented.

Funding acknowledgement: United States Department of Agriculture (USDA)

P243

Allele Alliance: Discovering the Optimal Path to Combine US and Chinese Maize Heterotic Groups

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Diversifying maize breeding germplasm into heterotic groups was one of the valuable breeding strategies that drove the dramatic increase of harvestable yield of US maize (*Zea mays*) hybrids, over the past 70 years. At the same time, maintaining adequate genetic diversity within modern heterotic groups is still required for continuous, long term yield improvement of newly developed hybrids.

Breeding of maize using heterotic groups was adopted in additional markets outside of the US including in China. However, the Chinese heterotic groups are unique, with limited overlap with the existing US heterotic groups. As such, the smooth introduction of breeding germplasm from the US to China is challenging.

Maize breeders for the Chinese market could benefit from an accurate measuring of allele distribution and frequency within and between US and Chinese heterotic groups. Such knowledge can assist in evaluating the breeding potential of each locus. To meet this aim, NRGENE has utilized its novel *GenoMAGIC*™ platform. This platform uses next generation sequencing (NGS) and novel algorithms to identify the allelic diversity in each locus.

NRGENE is using the *GenoMAGIC*™ platform to evaluate the potential of combining Ex-PVP US breeding material with Chinese germplasm. We find unique, as well as common alleles within and between US and Chinese representative genetic material. These findings allow us to single out a controlled path to combining genetic diversity from US and Chinese germplasm in order to achieve a significant increase in Chinese maize productivity.

P244

Analysis of candidate genes involved in drought tolerance in Mexican maize landrace Michoacán 21

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Improving productivity in drought conditions remains one of the major challenges for global agriculture. Michoacán 21 is a Mexican drought tolerant maize landrace, which exhibits "latency" - maintenance of the vegetative stage during drought stress and rapid recovery after re-watering. This cultivar has been evaluated by microarray analysis under water stress and after recovery irrigation (1), and found to show more drastic changes in global gene expression than a non-tolerant control. On the basis of this data, we have selected candidate genes for further analysis. Currently we are analyzing these genes by RT-qPCR to validate their pattern of expression and their possible relationship with drought tolerance.

To carry out the analysis of expression, plants of three cultivars: Michoacán 21 (drought-tolerant), B73 (drought-susceptible) and the hybrid (Michoacán 21 X B73) were grown under greenhouse conditions. Fifteen days after germination, drought conditions (-2 MPa in soil water potential) were initiated. Half of the plants were not watered and the rest were irrigated regularly. After the stress period, recovery irrigation was given and the plants were taken to the flowering stage. Samples of roots and leaves were collected in each stage (drought stressed plants, 24 hours after recovery irrigation and flowered plants with their respective well watered controls). In order to compare the physiological responses between stressed and non stressed plants the following parameters were measured: photosynthesis, stomatal conductance, leaf and soil water potentials and transpiration rate.

1. Hayano-Kanashiro, C.; Calderón-Vázquez, C.; Ibarra-Laclette, E.; Herrera-Estrella, L.; Simpson, J. 2009. Analysis of gene expression and physiological responses in three Mexican maize landraces under drought stress and recovery irrigation. PLoS ONE 4 (10): e 7531. doit:10.1371/Journal.pone.0007531.

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P245

Analysis of maize kernel density and volume with computed X-ray tomography single kernel near-infrared (NIR) spectroscopy

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Maize kernel hardness is an important trait for grain harvesting, processing, and end-product quality. However, modifying hardness through selection is difficult because methods for measuring kernel hardness are slow and labor intensive. One proxy for kernel hardness is kernel density. Kernel density measurements are typically determined by a pycnometer, which is a time consuming, bulk method and unable to account for air space isolated inside the kernel. We have developed a high-throughput method for accurate prediction of kernel density using a single-kernel near-infrared (NIR) spectroscopy. Analytical measurements of kernel density were derived using X-ray attenuation and computed tomography (CT) scans. The CT scanner produces high resolution radiographs from which whole kernel density and kernel material density, excluding air space, can be calculated. To build the prediction model, individual spectral profiles were collected from a set if diverse kernels using a single kernel NIR platform. Predictive models were built using partial least squares (PLS) regression of the NIR spectrum on the analytical values obtained from the CT scans. The predictive models performed well for both density traits with coefficients of determination (R^2) of 0.78 and 0.82 with prediction standard error terms well below the standard deviation of the trait distributions. The accuracy of the predictive model paired with the high-throughput capacity of the single kernel NIR platform will enable rapid selection of kernels with desired kernel hardness characteristics.

Funding acknowledgement: National Science Foundation (NSF)

P246

Association analysis of single nucleotide polymorphisms in candidate genes with root traits in maize (Zea mays L.) seedlings

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Root growth and development is not only critical for nitrogen acquisition in plants, but also to anchor the plant in the soil. Genes involved in maize root development have been isolated. Identification of SNPs associated with root traits would enable the selection of maize lines with better root architecture that might help to improve N uptake, and consequently plant growth particularly under N deficient conditions. In the presented study, an association study (AS) panel consisting of 74 maize inbreds were screened for seedling root traits in 6-, 10-, and 14-day-old seedlings. Allele re-sequencing of candidate root genes RTCL, RTH3, RUM1, and RUL1 was also carried in the AS panel lines. All four candidate genes displayed different levels of nucleotide diversity, haplotype diversity and linkage disequilibrium. Nucleotide diversity was highest in the RTCL gene (π=0.021), and lowest in RTH3 (π=0.007) and RUL1 (π=0.005) gene. When coding and non-coding regions within the genes were compared, nucleotide diversity varied across the genes.Gene based associaiton analysis was carried out between individual polymorphisms in candidate genes, and root traits measured in 6-, 10-, and 14-day-old maize seedlings. Association analysis revealed several polymorphisms within RTCL, RTH3, RUM1, and RUL1 genes associated with seedling root traits. These significantly associated SNPs also affected putative functional sequence motifs, mostly transcription factor binding sites, and major domains in the genes. Several nucleotide polymorphisms were significantly associated with seedling root traits in maize suggesting that all four tested genes are involved in the maize root development. Thus considerable allelic variation present in the root genes can be exploited for improving maize root characteristics. In current studies the same concepts are being utilized but on a larger scale using Genome Wide Association Mapping (GWAS) to study SNP associations with root architectural traits within the Ames Panel.

P247

Association Mapping Analysis for Drought and Aflatoxin in Maize using a Tropical and Sub-Tropical panel

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The major obstacles for corn producers in Texas are drought stress and aflatoxin infection caused by the fungus Aspergillus flavus. Previous research showed that maize lipoxigenase gene mutants, zmlox4-8::mu and zmlox5-3::mu exhibit greater resistance to drought stress and aflatoxin accumulation, respectively. Natural allelic variation at Zmlox4 and Zmlox5 was identified in a diversity panel comprising USDA Flint-Garcia/Buckler/Goodman and Williams/Warburton maize association mapping panels (400 lines). These lines were crossed to two LOX family knock-out-mutant isogenic lines in the Tx714 background (Tx714zmlox4-8::Mu/zmlox4-8::Mu and Tx714zmlox5-3::Mu/zmlox5-3::Mu). The hybrids (ZmLOX4/zmlox4-8::Mu) were evaluated under well-watered and drought conditions during 2011 and 2012. The hybrids (ZmLOX5/zmlox5-3::Mu) were evaluated under well-watered conditions, inoculated with Aspergillus flavus, and the aflatoxin content was determined. Grain yield and other important agronomic traits were collected for all the trials in this study. The exon 5 for zmLOX4 and zmLOX5 was sequenced for all the lines using the BigDye® Terminator method. In addition, genotyping of 346 lines was done using the Genotyping by Sequencing (GBS) method. A total of 62975 SNPs were called in the panel. A subset of SNPs with a low missing data rate (<7.5%) and low frequency imbalance between the two alleles (MAF>25%) was extracted to perform the genetic diversity and population structure analysis. Estimation of the variation components for the different trials indicated that useful genetic variation for QTL mapping is present for all the collected traits, except for moisture. Preliminary results have shown that high repeatability values were obtained for both grain yield (H2=0.63) and aflatoxin (H2=0.50). A promising result was the identification of a hybrid that yields 50% more than the commercial checks under severe drought and similarly under well irrigated condition. A candidate and whole genome association mapping analysis is underway for grain yield, aflatoxin accumulation and other important agronomic traits.

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P248

Association Mapping of Root Anatomical Traits in Maize (*Zea mays* L.)

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Root anatomical traits influence the acquisition and transport of water and nutrients, the metabolic cost of root growth and maintenance, and the mechanical strength of the root system. A number of experiments have shown significant benefits of root anatomical traits under biotic and abiotic stresses. Despite the high potential for improving crop performance and yield, few studies have been undertaken to characterize genetic variation and identify quantitative trait loci (QTL) for root anatomical traits. The main reasons possibly are the laborious nature of anatomical studies and the difficulty in properly extracting a large number of plant roots and accurately phenotyping the anatomical traits. In this study, we utilized RootScan, a semi automated image analysis for root sections to provide phenotypic data for a Genome-Wide Association Study (GWAS) to identify quantitative trait loci for root anatomical traits in diverse maize lines of the Wisconsin Diversity Panel. We have identified several significant Single Nucleotide Polymorphisms (SNPs) associated with root anatomical traits such as root cortical aerenchyma, metaxylem vessel area, number of cortical cell files, cell size, etc. The results will greatly improve our understanding of the genetic control of root anatomical traits. In addition, molecular markers associated with these traits may be useful in plant breeding programs by Marker-Assisted Selection.

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P249

Can verification of markers near known QTLs in different environments and genetic backgrounds be of practical use?

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Identification and development of markers for marker assisted selection to improve and create superior maize genotypes in developing eastern European countries is a rather challenging prospect. The major constrains are the limited resources for the developing and phenotyping mapping populations as well as a large number of markers required and the availability of biotechnology techniques suitable for high throughput genotyping. Based on the assumption that the markers which colocalise with QTLs that are stable across different environments and genetic backgrounds are the most informative and indicative for MAS, we selected fewer molecular markers already identify to be statistically associated with the QTL for the traits of interest in various mapping studies. To validate the marker trait associations and verify the stability of the QTLs in genetic background of Serbian inbred lines and in local growing conditions, initial set of 90 diverse inbred lines was chosen for genotyping with 40 microsatellite markers near already known QTLs and phenotyped in 5 environments for flowering time, height and yield components. The population structure was determined based on molecular date and the association analysis was performed using the program Tassel. Preliminary results showed that fewer highly polymorphic markers used in a carefully chosen set of inbreds with a range of phenotypic diversity can reveal significant marker trait associations stable in investigated environments, and that verification of markers could give more information about their practical use in maize breeding.

Funding acknowledgement: Serbian Ministry of Education, Science and Technological Development

P250

Characterization of the B73 x Ki3 recombinant inbred lines.

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Roots are the point of contact between soil and plants, and are responsible for both nutrient and water uptake from soil. The distribution of roots in soil has been shown to have a direct impact on the efficiency of the uptake. Although several different technologies have been used to explore the root system architecture (RSA), none were able to produce both high detail and high through phenotyping, and as such root research has been lacking. To solve this, we've previously developed and utilized a gel based system that solves those issues, and provides a method for imaging, phenotyping and analyzing 3D models of root systems over the course of several days. The data collected on the 26 different NAM founder lines, which represent the wide breath of variation available within maize, has shown a large amount of variation in the RSA of maize. We have since begun phenotyping one of the NAM RIL lines (B73 x Ki3) in the gel system, and are ultimately planning on performing a QTL analysis on the whole family. Preliminary data collected so far and presented here shows multiple segregating root traits, leading to a strong possibly of locating multiple QTL once the whole population is phenotyped.

Funding acknowledgement: National Science Foundation (NSF)

P251

Clustering of virus resistance genes in a multi-virus resistant maize line

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Virus diseases in maize can cause severe yield reductions threatening crop production and food supplies in some regions of the world. Genetic resistance to some, but not all, major virus diseases has been characterized and found to map to all maize chromosomes using different viruses, maize populations, environments and screening techniques. The maize inbred line, Oh1VI, is resistant to at least 8 viruses in different families. To determine the genetic architecture of virus resistance in this line, 256 recombinant inbred lines derived from a cross of Oh1VI and the virus-susceptible inbred line Oh28 were genotyped and screened for their responses to *Maize dwarf mosaic virus, Sugarcane mosaic virus, Wheat streak mosaic virus, Maize chlorotic dwarf virus, Maize fine streak virus, Maize mosaic virus, Maize rayado fino virus* and *Maize necrotic streak virus.* Composite interval mapping identified 21 resistance QTLs associated with the eight viruses. Of these, 15 were clustered on chromosomes 6, 3, and 10. Additional clusters of virus resistance QTLs were found in chromosomes 1 and 2. It is unknown whether these regions of clustered QTLs contain single or multiple virus resistance genes, but the linkage of genes conferring resistance to multiple virus diseases in this population could facilitate breeding efforts to develop multi-virus resistant crops.

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P252

Combining Ability Analysis of Expired Proprietary Short-Season Maize Lines

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Maize (Zea mays L.) is mostly produced as hybrid which is developed by crossing two inbred lines. Maize public and private inbred lines are under restricted use because they are protected by U.S. Patent and/or the U.S. Plant Variety Protection Act (PVPA). The patent expired inbred lines could serve as new breeding sources of cultivar development. Developing new early maturing maize hybrids is a long-term solution for maintaining profitable maize production under the cool and short growing northern U.S. environments particularly in North Dakota (ND). Incorporating expired- PVP lines from industry could provide unique combining ability for desirable traits. However, there is concern that these elite lines are at least 20 years old. The objectives of this research were to identify ex-PVP lines as breeding sources for short-season maize breeding programs and to identify unique hybrid combinations not tested before for economically important traits. Three groups of crosses were made for the study following North Carolina Mating Design II (Model I) including 12 released and experimental elite NDSU lines, 24 ex-PVP lines, and seven top industry testers. Progenies were produced in the 2010 North Dakota State University (NDSU) corn breeding summer nursery, Fargo, ND and in the 2010 - 2011 NDSU corn winter nursery in Pukekohe, New Zealand. Hybrids were planted in six different ND environments in 2011 and 2012 following partially balanced lattice experimental designs. Lp5 x ND2002, ND2007 x PHP02, ND2010 x PHP02, B14 Industry type x Q381, Iodent Industry typex CR1Ht, B14 Industry type x PHP02, LH205 x Iodent Industry type, and PHJ40 x B14 Industry type are few of the identified hybrids that exhibited higher yield, test weight, improved grain quality, low moisture (%), and higher lodging resistance compared to top checks. Several ex-PVP inbreds were also identified as promising in hybrids combination with both ND lines and industry testers.

Funding acknowledgement: North Dakota Corn Growers Association

P253

Comparative analysis of the inheritance of binary traits using phenotypic and molecular marker information

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This study aimed to compare inheritance patterns for oligogenic traits using phenotype and molecular marker information. A population was simulated that represented an F2 population derived from the cross between two contrasting homozygous parents. Simulated traits were controlled by one, two or three independent and linked genes. Molecular marker information was generated for the F2 individuals. This information was used to complement the information obtained from phenotypes, and to clarify the hypothesis tests in the case of dubious segregation within a concurrent hypotheses. Simulations included the parental genomes and F2 populations with 100, 200, 400 and 600 individuals. These individuals were phenotyped (0 or 1) and genotyped for five linkage groups. Each linkage group contained 11 co-dominant markers and 10 cM between markers. Quantitative trait loci detection was performed using a simple interval mapping methodology. Chi-Square tests, commonly used on classical genetics, failed on identifying correctly the pattern of inheritance for these traits controlled by linked genes. The tests also presented high error rates, mainly for small population sizes (100 to 200 individuals), when concurrent segregation hypotheses (percentage refers to false discovery rate- 13:3 and 3:1- 95%; 27:37 and 9:7- 50%; and 3:9:4 and 1:2:1-25%) were considered. Our results show the utility of molecular marker information for the identification of genes, and positioning and quantification of their effects on the expression of binary traits.

Funding acknowledgement: National Council for Scientific and Technological Development (CNPq)

P254

Current status of maize genetic resources in Serbia and their utilization in breeding

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The majority of maize genetic resources in Serbia are kept and maintained in the Maize Research Institute "Zemun Polje" (MRI) in Zemun and partly in the Institute of Field and Vegetable Crops (IFVCNS) in Novi Sad, two largest state-owned maize breeding institutions. The former is in charge of publically available Serbian maize gene bank comprising 5475 accessions, with 2217 landraces from former Yugoslavian countries and 3258 accessions of inbreds, landraces, advanced cultivars, synthetics, composites and varieties from 40 countries. Local landraces were characterized by 26 traits and classified in 18 agroecological groups: Montenegrin flints (224 varieties), Bosnian early dent (79), Kosmet flinty dents (101), Macedonian flints (115), Eight-rowed maize type of Northeastern America (103), Derived flints (101), Mediterranean flints (191), Small-kernelled flints (35), Eight-rowed soft dents (103), Romanian flints (85), Large-eared flint (88), Moravac (141), Dents type of US Corn Belt dents (152), Derived flints (99), Dents type of southern areas of US (101), Serbian dents (80), Flinty dents (123), Denty flints (148). The landraces such as Vukovarski Yellow Dent, Pecki Yellow Dent and Rumski Yellow Dent were the source for developing elite inbred lines due to their adaptability and good general combining ability and are still a valuable source of abiotic and biotic stress resistance and quality improvement. Montenegrin flints, Romanian flints and Eight-rowed maize type from MRI showed resistance to Fusarium sp., MDVD and corn borer, whereas Moravac and Serbian dents demonstrated resistance to Setospheria turcica. An extensive work to identify superior genotypes among the accessions maintained in Serbia resulted in developing genotypes with drought tolerance and good GCA, cytoplasmic male sterility, herbicide tolerance, stay green, high protein, starch and oil content and other purposes.

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P255

Diverse and different: sampling and evaluating 9,000 gametes from CIMYMTs maize landrace collection

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Maize was domesticated around 8,000 years ago in Mexico. Since then, it has been subject to farmer selection and improvement, and more recently to intensive breeding. Furthermore, most of modern breeding has been performed with a limited subset of the diversity present in the species. Landraces therefore represent an important resource for the potential discovery of novel beneficial alleles. The International Center of Maize and Wheat Improvement (CIMMYT) coordinates a Mexican Government funded initiative called Seeds of Discovery (SeeD) that aims at genetically and phenotypically characterizing a representative subset of their maize landrace collection. Analyses were performed to explore the genetic diversity and genotype-phenotype associations in this material.

The design used for SeeD is as follows: Individual plants for each of over 4,500 landrace accessions were planted. Leaf tissue was collected for each of the plants, and DNA was isolated and submitted for sequencing using Genotyping by Sequencing (GBS). The genotypic information was analyzed using a new version of the GBS pipeline, which is more sensitive to rare alleles. Over 2,300,00 SNPs were called using this approach. Each plant was also test crossed to one of 12 CIMMYT hybrids. The offspring of these crosses were phenotyped for several traits, including: days to anthesis; days to silking; plant height; ear height; root lodging; and stalk lodging. The phenotypic evaluation was done in 9 locations according to each accession's adaptation zone by CIMMYT and Mexican partner institutions. Genome Wide Association Studies for the previously mentioned traits were performed for the 2, 822 accessions that had both phenotypic and genotypic information as of mid 2012.

Landraces contain substantial genetic diversity compared to improved breeding material. The genetic architecture of different phenotypes; the amount of linkage disequilibrium; the density of markers, and the population genetics of a sample limit the performance of genotype-phenotype associations. Here, we explore the potential for performing Genome Wide Association in these material and highlight its limits and potential for improvement.

Funding acknowledgement: Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA)

P256

Do Provitamin A Carotenoids in Grain Affect Aspergillus Ear Rot Infection of Maize Hybrids?

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Breeding provitamin A-biofortified maize is an important strategy to help alleviate the widespread health problems associated with vitamin A deficiency (VAD). Besides the many negative human health impacts of VAD, consuming aflatoxin contaminated maize kernels is also a serious public health issue. Motivated by literature indicating that carotenoids protect against certain diseases, and that they interact with aflatoxin in fowl, rat and human models, we studied the correlation between provitamin A content and aflatoxin accumulation in maize kernels. Ten maize inbred lines varying in provitamin A content were crossed with three *Aspergillus flavus* resistant and three susceptible maize inbred lines using a North Carolina Design II, thereby obtaining four different hybrid groups: HiProA/Resistant (HR), HiProA/Susceptible (HS), LoProA/Resistant (LR), and LoProA/Susceptible (LS). F1 hybrid seeds were planted in replicated field trials at three locations: 1) Tlaltizapan (TL), 2) Agua Fria (AF), and 3) Mississippi (MS). About 14 days after flowering, artificial inoculation with *A. flavus* suspension was conducted by the side needle technique on the top ear of each plant. At harvest, inoculated ears were collected and visually scored for ear rot disease using a 1-5 scale: 1=healthy and 5=fully damaged. Preliminary results show no significant differences among the four hybrid groups for LSmean of % ear rot infection. However, negative correlations between B-cryptoxanthin and ear rot symptom scores were found at AF for the HR, HS, and LS hybrid groups, suggesting a role for B-cryptoxanthin on fungal growth inhibition. Evaluation of effects of ProvitaminA, environment, and GxE interaction will be presented. Also, role of provitamin A on Aspergillus ear rot inhibition will be discussed in the poster.

Funding acknowledgement: Harvest Plus Project

P257

Effect of genotypes with functional variation of asparagine-cycling on nitrogen utilization and ear growth

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Increasing nitrogen supplementation in maize positively affects asparagine concentrations throughout the plant, whereas this characteristic spike is not seen for other amino acids. This association of nitrogen application and asparagine suggests that asparagine plays a key role in nitrogen utilization. Illinois High Protein (IHP) also shows increased asparagine concentration when compared to other genotypes and exhibits a prolonged increase in ear growth in response to nitrate and vigorous ear growth under low nitrogen conditions. Reciprocally, Illinois Low Protein (ILP) has a very low concentration of asparagine and is much poorer in its nitrogen uptake and utilization. IHP and ILP were utilized as testers crossed to a diverse set of inbreds (NAM parents and ex-PVP inbreds) to analyze their effect on amino acid concentrations as well as other physiological traits. Hybrids with the IHP tester had on average nearly four times the asparagine concentration in earshoot tissue at anthesis compared to the corresponding inbred crossed to ILP. Also, stover N percentage was almost always higher in the ILP hybrids, whereas the IHP hybrids always had a higher grain N percentage, suggesting superior N remobilization conferred by IHP. Illinois Reverse High Protein (IRHP) was derived from IHP but has the low protein characteristic of ILP. IHP and IRHP have functional variants of the asparagine-cycling genes Asparagine Synthetase (AS) and Asparaginase (ASNase). Using a population derived from the cross of IRHP and IHP, we examined the effect of these two genes on ear growth and grain protein. Increasing AS expression was associated with increased grain protein concentration, whereas reducing ASNase activity was associated with decreased ear size and weight. These results indicate that the two possible routes to increasing plant asparagine have distinct and opposing effects on the grain yield-protein relationship.

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

P258

Effectiveness of line per se performance trials for drought tolerance screening in tropical maize

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To optimize the efficiency of maize drought breeding, the ability to predict testcross performance (TP) under drought stress using line *per se* performance (LP) of the parental inbreds would be useful. We evaluated LP and TP of tropical inbreds in well-watered and drought environments in Kenya and Mexico. Our main objective was to determine if LP under drought stress was predictive of TP for grain yield under drought stress, and if selection for LP under drought stress would result in reduced yield potential for TP under well-watered conditions. Average yield reduction under drought stress was 77% for lines and 68% for testcrosses. Average genotypic correlations between lines and testcrosses under drought stress were positive and low (rg = 0.48), but correlations increased with increasing levels of drought stress in both LP and TP trials. Averaged over all sets, indirect selection for LP was predicted to be only 57% as effective as direct selection for TP under drought stress, but was on average substantially higher in testcross sets where yield reduction due to drought was 70% or more. Thus, LP under drought stress could be used to develop hybrids for severely drought prone environments. Moreover, LP under drought stress was uncorrelated with TP for grain yield under well-watered conditions, showing that selection of lines *per se* for drought tolerance would likely not reduce yield potential of testcrosses.

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P259

Environmental and genetic dissection of flowering time in a population subjected to a decade of temperature adaptation

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Exotic maize germplasm is an important source of allelic diversity for N. American maize improvement. Exotic germplasm represents only a small fraction of the total parentage of U.S. cultivated maize. In a USDA-NIFA funded project, called Maize ATLAS (Adaptation Through Latitudinal Artificial Selection; http://www.maizeatlas.org/), our team is developing approaches to study the genetic basis of response to artificial selection underlying environmental adaptation. In this study, a tropical landrace, Tusón, adapted over a decade of selection for earliness to a temperate environment was evaluated in a multi-environment trial. Phenotypic data on 300 families was collected from trials at nine locations representing a latitudinal transect from Wisconsin to Puerto Rico. The among-environment variation was 20X greater than the within-environment variation in flowering time, with temperature as a primary driver of the variability. A significant but relatively small amount of the variation was associated with GxE interactions and the overall response to selection for flowering time was consistent across environments. An experimental approach combining association mapping with analysis of allele frequency change is being used to dissect the genetic basis of flowering time and the response of flowering time QTL to artificial selection for adaptation.

Funding acknowledgement: United States Department of Agriculture (USDA)

P260

Evaluation of the seed vigor and germination of two haploid inducing lines and introduction of a simple, quick and practical method for determining seed viability.

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Haploid inducing lines are widely used in the production of double haploids (DH) in maize. But problems can occur with the germination and vigor of some these lines. Haploid inducing lines, RWS/RWK-76 and RWK-76/RWS, were examined for viability by Tetrazolium (TZ) and standard germination testing. Each experiment included four replications with 25 seeds in each replication. The TZ results indicated that 59% of the seeds of RWK-76/RWS were viable, compared with only 12% in RWS/RWK-76. Similarly, the percent and speed of germination in RWK-76/RWS (25%, 1.53) was lower than RWS/RWK-76 (74%, 4.30). For determining seed viability with a quick method, the TZ test was repeated in a different way. Seeds from each replicate from each genotype were placed into beakers filled with distilled water. The seeds would either float or sink. Subsequent TZ testing confirmed that seed that floated were dead, and seeds that sank were alive, although some of them had defective embryos. The dead seeds in both genotypes failed to develop an embryo, leaving an empty cavity that would fill with air and cause to float seeds on the water. So we introduce a simple and practical method to separate living seeds from dead ones. In addition, we were looking for genomic regions affecting germination.

P261

Evaluation of Viability in Subnormal Maize Kernels using Near-infrared Spectroscopy

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In vivo induction of maternal haploids in maize, other than normal haploids and diploids, subnormal kernels which had defective embryos or endosperms or both were also obtained. Through the ploidy level determination, more than 30% haploids among all the germinated subnormal kernels were found, however, the germination percentage of subnormal kernels is below 40%. In order to further evacuate its utilization value and improve the efficiency, a fast and non-destructive approach to select the subnormal kernels with germination ability has been developed by near-infrared spectroscopy. The discrimination models were established and validated with 600 spectral samples from 200 subnormal kernels, half of which germinated within 3 days through the standard germination test and the other half did not germinate within 7 days. The spectra were collected by a Fourier transform spectrometer in the diffuse reflectance mode. The feature wavelengths which could explain the germination ability of subnormal kernels were selected via the methods based on Fisher's ratio, Kolmogorov-Smirnov test, and Uninformative variable elimination, respectively. The results show the feature wavelengths selected by different methods all distribute in the similar range (1100-1460nm and 1820-2490nm). The range above contains the absorbance peaks of multiple components, such as oil, starch, protein, and cellulose. The discrimination models were established based on the partial least squares method and evaluated through Monte Carlo cross validation. The correct discrimination rates for the seeds with germination ability and the seeds with no germination ability arrive at 92.2% and 84.9%, respectively. The test time of each kernel is about 30 seconds.

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P262

Experimental design for optimal detection of QTL x environmental interaction: example of maize performance across a phosphorus gradient

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The development of any organism is controlled by a network of genes (QTL) as well as by environmental factors. For this reason, the genetic study of quantitative or complex traits has long been one of the most daunting tasks in biology. Phosphorous (P) is essential for plant growth, and P limitation is among the major constraints on global plant productivity.
The capacity of a plant to access soil P varies among species and genotype; for example, previous results have a difference in tolerance to growth under low P between B73 and Mo17. The objective of this study is to address the question of optimality of experimental design for the detection of QTL by environment effects. We will use a computational approach to simulate the number, distribution and sizes of effect and interaction of QTLs affecting phenotypic variables in two RIL populations of maize with known marker genotypes. Under different QTL scenarios, we will determine which experimental design gives the best estimation of the parameters of interest. Comparing the results of the analyses with the known simulated parameters, we will determine the optimal design under a number of QTL scenarios. We are going to select the one that allows us to detect the largest number of QTL, reducing the false positives rate. Another parameter that we can to optimize is line selection based on measures of genetic dissimilarity. The aim of this study is to arrive at a suitable design for efficient detection of QTL x phosphate-availability effects in a greenhouse experiment, using the IBM RIL collection.

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P263

Exploring natural variation underlying R gene-mediated immune response in maize using MAGIC

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This project seeks to unravel the genetic architecture of the hypersensitive response (HR), a most common yet complex defense response of the plants. Despite several previous studies using mutagenesis screens in different plant species, knowledge regarding this phenomenon is still limited. We have devised an effective method, Mutant-Assisted Gene Identification and Characterization (MAGIC), to explore the extent and nature of natural variation capable of impacting the maize HR defense response. This method makes use of the phenotype of a mutant (for a gene affecting the trait of interest) as a reporter to identify interacting genes present naturally in diverse germplasm. A constitutively-expressed mutant allele of Rp1 disease resistant gene, Rp1-D21, causes spontaneous induction of HR lesions even in the absence of a pathogen. Using the phenotype of Rp1-D21 as a reporter of HR response, it has been observed that remarkable amount of variation exists in the maize germplasm that can enhance or suppress the expression of HR. When conducted on the IBM RIL population, this approach led to the identification of a large QTL, which we have named Hrml-1 (for HR modulating locus-1). Encouraged by the success of this study, we have started to integrate MAGIC with the NAM (Nested Association Mapping) resource. NAM is a collection of 5,000 RILs derived from a cross of B73 with 25 diverse maize inbred lines (called NAM founders), with each cross yielding 200 RILs. The F1 testcross progenies obtained from the cross between Rp1-D21 and NAM RILs were evaluated in two field locations over two field seasons. Composite interval mapping analysis for individual sub-populations identified a total of 33 significant QTL. Identity of the genes underlying the identified QTL will be confirmed by a combination of targeted EMS mutagenesis and transposon tagging techniques.

Funding acknowledgement: National Science Foundation (NSF)

P264

Extensive Genetic Diversity and Low Linkage Disequilibrium within the COMT Locus in Germplasm Enhancement of Maize Populations

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The Caffeic acid 3-O-methytransferase (COMT) gene is a prime candidate for cell wall digestibility improvement based on the characterization of brown midrib-3 mutants. We compared the genetic diversity and linkage disequilibrium at COMT locus between populations sampled within the Germplasm Enhancement of Maize (GEM) Project and 70 elite lines. In total, we investigated 55 exotic alleles from the GEM Project at the COMT locus, and discovered more than 400 polymorphisms in a 2.2 kb region. The pairwise nucleotide diversity (π) for the exotic alleles of COMT gene was 0.0172, and much higher than the reported pairwise nucleotide diversity of various genes in elite inbred lines (π=0.0047 to 0.0067). At this locus, the average number of nucleotide differences between any two randomly selected alleles was 27.7 for exotic populations, much higher than 18.0 for elite lines. The ratio of non-synonymous to synonymous SNPs was 3:1 in exotic populations, and significantly higher than the 1:1 ratio for elite lines. Selection tests detected selection signature in this gene in both pools. But different evolution patterns from progenitor to landraces and from landrace to inbred lines were suggested by significant negative and positive neutral test statistics. The linkage disequilibrium (LD) decay in exotic populations was at least four times more rapid than for elite lines with r2 > 0.1 persisting only up to 100 bp. In conclusion, the alleles sampled in the GEM Project offer a valuable genetic resource to broaden genetic variation for the COMT gene, and likely other genes, in elite background. Moreover, the low linkage disequilibrium makes this material suitable for high resolution association analyses.

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

P265

Extreme early flowering of *Zea perennis*, *Zea diploperennis*, and *Zea luxurians* under 24-hour light regime

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Under native conditions, the day-sensitive species *Zea perennis*, *Z. diploperennis*, and *Z. luxurians* flower six to nine months after being planted from seed. However, we observed flowering in these species two months—a quarter of the normal time—after planting. Seeds were planted in a growth chamber under the following conditions: full light with the balance shifted to red light, 30 degrees Celsius, and low humidity. All of these conditions were held constant for 24 hours until early flowering was observed. Aside from stunted growth, plants appeared normal. We hypothesize that when all diurnal cues are removed from these three species, an autonomous flowering process becomes activated and triggers abnormally early flowering.

P266

Genetic analysis of kernel elemental composition (ionome) profiles in the maize Nested Association Mapping (NAM) population

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Kernel ionomic profiles are highly heritable indicators of genetic and environmental influences on elemental uptake and accumulation in maize. This study aims at estimating the heritability and genetic correlations as well as identifying the chromosomal loci that are associated with different mineral ions that constitute the plant ionome. A high throughput ionomics profiling system was developed to measure the concentration of the 20 elements in more than 25,000 single maize kernels. The study is based on the diverse maize Nested Association Mapping (NAM) population grown by the Maize Diversity Project group at four different locations (New York, North Carolina, Florida and Puerto Rico) in 2006. Our results from this high precision phenotyping indicate a wide range for heritability estimates from Cobalt ($h2 = 0.17$, $p < 0.05$) to Molybdenum ($h2 = 0.89$) with most of the traits being moderately heritable. There is also variability among the 27 NAM families for the different mineral elements. Genetic correlations among some traits are also high, confirming known shared physiological pathways. Nested association mapping with the HapMapv2 SNPs should reveal the power of this approach in dissecting the genetic architecture and identifying genetic factors that determine the maize grain ionome.

Funding acknowledgement: National Science Foundation (NSF)

P267

Genetic architecture of nitrogen utilization efficiency in the maize intermated B73 x Mo17 recombinant inbred line high-resolution genetic mapping population

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Genetic improvements in nitrogen utilization efficiency (NUtE) bring significant potentials to crop production both economically and environmentally. Previous efforts to decipher NUtE architecture in maize (*Zea mays*, L.) have been compromised by the complexity of this trait as well as the lack of advanced technology platforms. The objectives of this research were to identify quantitative genetic loci (QTL) controlling NUtE in maize using the high resolution intermated B73 × Mo17 recombinant inbred line (IBMRIL) population, with the ultimate goal of discovering the underlying causal genes through genotyping-by-sequencing (GBS) enabled fine mapping. Altogether across 13 traits and two years, 160 QTL were identified in this study. QTL that were consistently significant in both 2006 and 2007 were integrated into nine consensus genomic regions distributed over six chromosomes. After saturating the regions with dense single nucleotide polymorphism (SNP) markers generated from next generation sequencing on the IBMRIL population, seven regions were successfully fine-mapped to a few candidate genes. Promising candidates include a HVA22 under a QTL on chromosome 9 affecting grain N and NUtE, and a GLYCOSYLTRANSFERASE under a QTL on chromosome 6 influencing grain N concentration. NUtE candidates from GBS fine mapping were found to be involved in various physiological pathways, revealing the composite nature and complex framework of maize NUtE. Results from this research provide insights into the genetic architecture of maize NUtE, facilitating marker assisted selection and positional cloning of the QTL in the future.

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P268

Genetic Dissection of Quantitative Traits Using a Bulk and Resequencing Method on a Large Segregating Population of Maize

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Bulk Segregant Analysis (BSA) is commonly used for the characterization of genes with major effects. The availability of high density genotyping technologies have allowed for the identification of single nucleotide polymorphisms (SNPs) that could be associated with phenotypes of interest in collections of diverse germplasm. The primary aim of this project was to couple these two methodologies for the identification of causative SNPs contributing to quantitative traits using phenotypic extremes from large segregating populations. As a proof of concept, 10,000 individual segregating maize plants from the inter-mated B73 X Mo17 Syn14 population were grown in one environment alongside 224 recombinant inbred lines (RIL) derived from the Syn 4 IBM population. Flowering time, measured in growing degree days to pollen shed, and plant height were recorded for extreme individuals in the Syn14 population and all of the IBM RILs. BSA was performed using whole genome resequencing data from pooled DNA of the 46 most extreme plants for each distributional tail of the Syn14 population for each trait measured. Additionally, QTL mapping was performed on the IBM RILs for both flowering time and plant height. Observed phenotypic distributions for both traits measured in these two populations suggest a potential presence of dominance. Using the BSA-resequencing method, two putative regions contributing to flowering time, and six putative regions contributing to plant height were identified. The number of regions found using BSA-resequencing was similar to the number found using traditional QTL mapping. While no overlapping regions were identified between the two mapping methods for plant height, one overlapping region was found for flowering time on chromosome 8, a region previously shown to contain Vgt1 (Vegetative to generative transition 1). Our results suggest that BSA-resequencing has the potential to rapidly identify genetic regions contributing to quantitative traits.

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P269

Genetic regulation of agronomic traits in maize

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Complex agronomic traits in maize are often considered to be controlled by multiple loci with minute effects, but the genetic determinations of most of the agronomic traits are poorly understood. Here, we report our result of genome wide association studies (GWAS) of a template maize population, with most of lines from elite breeding germplasm from China and USA. A total of 66 million SNPs were identified by re-sequencing ~700 maize inbred lines. Genome-wide association studies for 20 agronomic traits were performed. Taking a non-regression liner model, 214 loci were identified as candidate regions and they could explain 22% of the phenotype variance on average. Among all the identified candidate loci, 7 included previously cloned genes or known functional polymorphisms for three traits. Due to the nature of quick linkage disequilibrium (LD) decay in maize population, 81 candidate loci were found contain only one gene. Very interestingly, 86 loci were found to limited in regions that contain no protein-coding sequences, indicating that these noncoding sequences may have contributed to the phenotypic variation of the agronomic traits studied. Our studies demonstrated that GWAS in maize can reach an unprecedented resolution if provided enough SNPs makers along the genome. Whole genome low coverage re-sequencings can be an effective genotyping strategy for GWAS in maize breeding population.

P270

Genetic variability in tropical maize breeding populations subjected to a reciprocal recurrent selection program

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Reciprocal recurrent selection (RRS) is an important breeding procedure commonly used to improve populations' combining abilities and performance without dramatic loss of genetic diversity. It is often important to monitor the change in diversity and genetic gain to ensure continuous genetic improvement.

Two complementary tropical maize breeding populations (AT1 and AT2) were synthesized from diverse progenitor lines and maintained under recurrent selection programs. To analyse the loss of genetic diversity due to the effect of recurrent selection, thirty randomly selected individuals from the progenitors (p) and four selection cycles (C0, C3, C6 and C11) were assessed using 15 polymorphic SSR markers. Genetic diversity estimates (He), allelic richness and Nei's genetic distance were estimated. As a result of recurrent selections, genetic diversity averaged over loci, declined from unselected cycle (C0) to advanced cycles (C6 and C11) in both breeding populations. About 33 and 25% of allelic richness were lost as selection advanced from C0 to C11 cycles in AT1 and AT2, populations respectively.

Wright's fixation index (FIS) was estimated to quantify the deficiency or excess of the heterozygosity in the breeding populations' different selection cycles. In both populations, the C0 cycles were characterized by a slight excess of heterozygotes whereas; the progenitors and the advanced cycles (C6 and C11) were characterized by excess of homozygotes. Clustering of the populations based on Nei's genetic distance showed clear separation of selection cycles indicating that the recurrent selection process resulted in the differentiation of populations.

This study demonstrated that by using limited informative markers the loss of genetic variability can be monitored. This information can be used either to infuse new germplasm or increase the population size to minimize the reduction of genetic diversity.

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P271

Genetic variation for high temperature tolerance in maize

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As global warming becomes inevitable, the sustainability of agricultural production in US and worldwide faces serious threat from extreme weather conditions, such as drought and high temperature (heat) stresses. While drought stress can be alleviated through irrigation, little can be done with high temperature stress through crop management. More importantly, high temperature induced tissue injuries in maize are mostly irreversible; and a few days of temperature above optimum at pollination can result in significant yield loss. Therefore, the only feasible way to cope with temperature extremes in maize production is through genetic improvement of high temperature tolerance in maize breeding lines. We have taken a genetic approach to study of genetic mechanisms of high temperature tolerance in maize. Maize inbred lines with contrasting phenotypes for high temperature tolerance were used to generate mapping populations. Preliminary genetic analysis revealed several independent traits that contribute to the variation for high temperature tolerance in field-grown maize. Several major QTLs associated with high temperature tolerance were identified in 2 of the NAMs RIL populations. In addition, genetic variation among 537 inbred was evaluated for vegetative and reproductive tissue high temperature tolerance traits in field condition. Genome-wide association analysis will be performed to identify chromosome regions and/or genetic loci contributing to high temperature tolerance in maize.

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P272

Genomewide prediction accuracy within 1000 biparental maize populations

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In genomewide selection, the expected correlation (r_{MG}) between predicted genotypic values and true genotypic values has been derived by Daetwyler et al. as a function of the training population size (N), trait heritability (h^2), and effective number of quantitative trait loci (QTL) or chromosome segments affecting the trait (M_e). Our objective was to evaluate the accuracy of genomewide selection in 1000 biparental maize breeding populations and compare the observed accuracy with the expected r_{MG}. Genomewide prediction was by ridge regression-best linear unbiased prediction and the observed r_{MG} was obtained by dividing the correlation between marker-predicted values and phenotypic values by the square root of h^2. We also modified Daetwyler et al.'s equation for expected r_{MG} to account for recombination between a QTL and a marker. Across the 1000 populations, the mean and range (in parentheses) of observed r_{MG} was 0.24 (–0.32, 0.77) for grain yield, 0.41 (–0.23, 0.90) for grain moisture, and 0.38 (–0.28, 0.95) for test weight. The h^2 and N had the largest influence on observed rMG. The expected rMG from Daetwyler et al. was much higher than the observed r_{MG}. In contrast, the observed r_{MG} values were centered around the expected r_{MG} based on imperfect linkage between a QTL and marker, although the spread of the observed rMG was still very large. We conclude that predicting the accuracy of genomewide predictions is difficult.

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P273

Genomewide prediction within an untested biparental cross

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Maize breeders develop new inbreds by crossing two parents (A and B) and selecting the best inbreds from the A/B cross based on their performance when crossed to a tester. It is helpful to predict the testcross performance of A/B progeny prior to phenotyping the population. Our objective was to determine the prediction accuracy and gain from selection using different populations as a training set for an A/B cross. Suppose * refers to any inbred that belongs to the same heterotic group as A and B, and that the same tester is used for all populations. For 30 different A/B crosses, we compared genomewide predictions from three training populations: (i) A/B itself ("Within"); (ii) A/* and */B crosses (general combining ability or "GCA"); and (iii) */* crosses ("Control"). In the GCA model, 4 to 38 A/* and */B crosses (634 to 5766 individuals) were used as the training set for each A/B cross. For grain yield, the mean prediction accuracies obtained by ridge regression best linear unbiased prediction for each of the three methods (in parentheses) were 0.14 (Within), 0.18 (GCA), and 0.06 (Control). For moisture, the mean prediction accuracies were 0.34 (Within), 0.37 (GCA) and 0.07 (Control). For test weight, the mean prediction accuracies were 0.25 (Within), 0.28 (GCA), and 0.06 (Control). The prediction accuracies as well as gains from selection indicated that the GCA model is a suitable method for designing training populations for prediction within an untested biparental cross.

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P274

Genomic Approaches for Improving Grain Yield in Maize Using Formerly Plant Variety Protected Germplam

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The public availability of commercially-derived inbreds formerly protected through Plant Variety Protection (ex-PVP) provide enhanced germplasm that can make public research more valuable and adaptable to private sector breeding efforts. The objectives of this study were to demonstrate the utility of ex-PVP material by 1) characterizing available ex-PVP lines in a diallel 2) identifying QTL stable across testers for yield and yield components in ex-PVP-derived F2:3 and reciprocal advanced backcross (RAB) populations, and 3) comparing the accuracies of genomic selection and phenotypic selection in an F2:3 breeding population. We identified B73, PHG84, LH123HT, and PHZ51 as inbreds with high general combining ability for yield in a half-diallel with 10 ex-PVP inbreds and two public inbreds. LH51xPHG35 was used to derive an F2:3 population that was testcrossed to two ex-PVP testers and a contemporary Dow Agrosciences (DAS) tester and evaluated for yield and yield components. Over 65% of the DAS-testcrossed lines ranked within commercial check performance. Yield QTL stable across multiple testers were identified on chromosomes one and seven. Additional yield QTL were identified on chromosomes three and ten using a new approach termed RAB QTL mapping (RAB-QTL) using a RAB population derived from LH51xPHG35. High yielding lines that can be further enhanced by selecting for all of the yield QTL were developed through RAB-QTL. Using RAB-QTL improved the ability to detect QTL over traditional advanced backcross QTL mapping by equalizing allele frequencies and allowing for a contrast between homozygous marker classes. The LH51xPHG35 F2:3 population was also used to compare the accuracy of phenotypic and genomic selection using 2010/2011 data to predict the performance of a mixture of tested and untested lines. Predictions were subsequently validated in 2012 yield trials. We concluded that genomic and phenotypic selection performed equally well which may have significant implications in early generation testing methodologies.

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P275

Genomic impact of artificial selection for number of ears per plant in maize

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Artificial selection increases the frequency of favorable alleles in a population. When selection is strong and the population size is large, changes in allele frequency at selected loci may be markedly greater in magnitude than at neutral loci. The objective of this work was to track allele frequency changes at SNP loci in an artificially selected population in order to identify and assess those that were impacted by selection. The Golden Glow maize population was selected for thirty generations for an increase in the number of ears per plant. After thirty generations of intense selection (selection intensity=1%-3%), with an approximate effective population size of 571 individuals, the population exhibited a more than threefold increase in number of ears per plant. Sequencing of 48 pooled individuals from the base and selected populations was conducted to a target of 50X coverage. After alignment, usable coverage was approximately 9.8X. The significance of allele frequency change at each SNP locus was evaluated using a sliding window approach based on FST; single-locus FST values were computed and subsequently averaged over 25-SNP sliding windows to reduce random sampling error. Significance was assessed based on a 99.9% empirical threshold. Twenty eight regions with a significant signal for selection were identified. These regions ranged in size from under 5 kb to 9.2 Mb, with a median size of 69.3 kb. From 0 to 21 genes were within each selected region, providing excellent resolution for gene detection. An evaluation of QTL that are suggested by this scan is reported, along with the empirical resolution of such a scan with respect to isolating genes for future study. Preliminary findings suggest that the majority of selection operated on alleles with initially intermediate frequency, and few selected sites show evidence of being driven to near fixation.

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P276

Genotype by Environment Interaction of Sorghum Flowering Time

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Flowering time is a complex trait that controls adaptation of plants to their local environment. The initiation of flowering in plants is controlled by endogenous and environmental signals. Sorghum is a short-day tropical species that exhibits substantial photoperiod sensitivity and delayed flowering in long days. Genotypes with reduced photoperiod sensitivity enabled sorghum's utilization as a grain crop in temperate zones worldwide. In this study, we are trying to investigate the G × E interaction of sorghum flowering time and provide possible solutions to tackle this issue in genetics research and plant breeding. Flowering times of 250 entries from a sorghum recombination inbred line (RIL) population were phenotyped. We observed significant G × E interaction for this trait. In Puerto Rico, a short day environment, all entries in this population flowered in a 20 days period. However, in Manhattan KS, a long day environment, the range of flowering times extended to approximately 70 days. Some photoperiod sensitive entries flowered early in short day environment, but extremely late in long day environment. This population was genotyped using genotyping by sequencing (GBS). Two QTLs located on chromosome 6 and 10 explained 45% and 12% of the phenotypic variation, respectively. These two QTLs showed QTL × QTL interaction in long day environment. Finding the genome locations of the underlying genes could provide valuable information for marker assisted sorghum breeding.

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P277

Genotyping-By-Sequencing (GBS): Building A Resource for the Maize Community

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Genotyping-by-Sequencing (GBS) is a simple, inexpensive method to produce reduced representation libraries suitable for sequencing on the Illumina platform and the bioinformatics software used to process the resulting sequence data. Together, these two pieces comprise a system for producing millions of high quality molecular markers in high diversity species such as maize. In 2012, we made significant improvements to expand the capacity of the GBS analysis pipeline and support software. We analyzed 33,000 maize samples together to produce over 2 million high quality single nucleotide polymorphism (SNP) markers. The samples processed include the US NAM, Chinese NAM, many lines from the Ames collection, numerous land races from CIMMYT and many others. Computational resources used, results of diagnostics performed, allele frequency distribution and access to the data are discussed.

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

P278

Genotyping-by-sequencing the genomic imprints of temperate adaptation in maize landraces from the southwestern United States and northern Mexico

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Beginning approximately 4,000 years ago, the southwestern United States provided the geographic and cultural context for temperate adaptation in maize as the crop moved northward from central Mexico. American Indian communities in the region have deep roots and traditional farming practices, including germplasm curation and the continued use of early agricultural sites through the present.

Using genotyping by sequencing (GBS), we genotyped multiple individuals from 38 southwestern United States landraces. This GBS build was designed to identify large numbers of rare alleles by comparing thousands of maize inbreds and landraces. Overall, 2.1 million SNPs were identified, although coverage per individual is substantially lower. We compare results from these tests with comparable analyses in the highly diverse Ames inbred panel, and the CIMMYT SeeD panel of tropical and Andean landraces.

Many of the flowering time and cold temperature adaptations evolved in this region, we expect the modern southwestern maize landraces to vary between highland and lowland accessions and test these groupings for statistically significant differences in allelic variation. Additionally, since flowering time – a well understood trait characterized by many common QTL of small effect but with significant allelic variation – is critical for successful temperate adaptation and we focus analyses on genomic regions with known flowering time QTL. We evaluate patterns of selection at these loci using multiple methods including a characterization of the haplotypes in the vicinity of flowering time QTL and compare them with patterns of selection across the rest of the genome. Additionally, the inter- and intra-population dynamics of upland and lowland differentiation are studied.

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

P279

Heat tolerance of maize - Improving the tolerance by next generation plant science

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Due to climate change, the number and intensity of heat waves in Central Europe is expected to increase. This in turn will affect maize cultivation. Especially anther fertility and the coordination of male and female flowering are disturbed by heat stress. Further, young seedlings can be impaired when maize is sown as a biomass crop after cereals in a hot summer period. Our project aims to identify candidate genes for heat tolerance of maize by transcriptome profiling and to identify molecular markers linked to heat tolerance traits by QTL mapping. Results should facilitate breeding of heat tolerant maize varieties.

The first part of the project consisted in comparing eight European flint and dent inbred lines concerning their reaction upon heat stress. Phenotyping was performed in growth chambers at three temperature levels (25°C, 32°C and 38°C) during seedling stage. Transcriptomic variation between genotypes and stress intensity was revealed on a molecular level by RNA sequencing. Promising candidate genes with differential expression at heat stress compared to normal temperature will further be validated by qRT-PCR.

In the second part of the project, segregating populations with 600 genotypes were developed out of the aforementioned inbred lines. They were phenotyped in growth chambers under the same conditions as their parental genotypes. The populations show a high degree of genetic diversity for heat tolerance. Additionally, phenotyping was done at the field level at two heat stress locations in Southern Europe and at two standard locations in Germany by the plant breeding companies KWS and Limagrain. Genotyping was performed by RAD sequencing. The obtained markers together with experimental results will be used to develop a QTL map assisting breeding companies to evaluate their breeding material.

Finally both parts of the project will be combined by projecting the candidate genes on the genomic map.

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P280

High resolution QTL detection using large F2 population and high throughput genotyping in maize

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High-density molecular marker maps facilitate the dissection of genetic bases for complex traits through quantitative trait locus (QTL) analysis with high accuracy. Recent advances in next-generation sequencing technologies have provided cost effective platform for high-quality single nucleotide polymorphisms (SNP) detection for constructing high-density genetic map. Here, we developed a set of 752 F2 segregating population derived from inbred lines C72 and 787, which showed significant differences in tassel branch number. Using genotyping-by-sequencing technology, low-coverage (average 0.1X) sequences were generated for individuals of the F2 population to construct a high-density SNP map by sliding window approach. Two traits, tassel branch number and silk color, were scored, and multiple QTL mapping tools in R/qtl were used to detect the QTLs for both traits. With only 238 individuals, our analysis identified 5 QTLs controlling tassel branch number, and one of them falls over ramosa 1 on chromosome 7; the rest of the QTLs dispersed on chr3, chr4, chr9 and chr10, where the smallest interval of the QTLs was ~300-kb and encompassed 14 known genes. Validating the position of r1 for silk color was used to assess the quality of the map. Our method provides new strategy for quick identification of QTLs in crop.

P281

High Throughput Phenotyping - A boost for genomics in the 21st century

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Due to the development of highly automated genetic analysis, plant genomics has immensely enlarged our understanding of the genetic structure of plants over the last two decades.
The fast evolving need to identify interactions between genes and environmental factors (biotic and abiotic) that brings about a certain plant phenome made it necessary to develop quantitative, reproducible and highly automated plant phenotyping systems for large plant numbers.
Phenotyping systems such as these have to integrate reproducible plant management (randomization, watering) and comprehensive imaging of root and shoot far beyond human vision (visible light, fluorescence, near infrared, infrared, X-rays, THz) as well additional chemical analysis methods. Immediate and automated image analysis of the stored images and further data transformation using plant shape and plant growth models are the important intermediate steps before undertaking statistical data analysis of the phenotyping results to characterize plant phenotypes quantitatively. Such quantitative data contributes in a decisive way to the further analysis of gene functions (tilling, QTL etc.), especially under fluctuating or stress-induced environmental conditions with a special focus on complex traits like yield or drought tolerance.
This presentation will provide a survey on phenotyping technology and the close interaction between phenotyping technologies, modeling approaches and the new opportunities of fast and automated high-throughput genomics.

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P282

High-resolution mapping of tocochromanol and carotenoid grain traits via NAM-GWAS reveals distinct genetic architectures in maize

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For millions of people in developing countries, plant-based foods are the major source of provitamin A carotenoids and vitamin E tocochromanols. More than 125 million preschool-aged children are vitamin A deficient in developing countries, with an estimated 250,000 to 500,000 of them becoming blind every year. Although clinical vitamin E deficiency is rare, suboptimal dietary intake of vitamin E at levels that are associated with an increased risk for cardiovascular disease have been reported in specific population segments of the US and developing countries. Maize is an important staple crop in many of the countries where these nutritional deficiencies are present and has considerable genetic variation for carotenoid and tocochromanol grain levels. However, the varieties of maize grain typically used for human consumption do not provide adequate daily levels of provitamin A and vitamin E. We determined the genetic basis of more than 40 carotenoid and tocochromanol grain traits through a genome-wide association study of the maize nested association mapping panel. While a substantial proportion of QTL identified for carotenoids coincided with the genomic position of known carotenoid biosynthetic pathway genes, most of the tocochromanol-related QTL were not associated with known genes from the tocochromanol biosynthetic pathway. Even though all of these biochemical grain traits were highly heritable, a greater number of QTL were identified for tocochromanols relative to carotenoids. Taken together, these results suggest a simpler genetic architecture for carotenoid traits that is possibly due to the recent evolution of this pathway in maize endosperm. Notably, many of the QTL identified for carotenoids were also associated with orange kernel color intensity, implying that visual selection for dark orange kernel color could be enhanced with allele-specific marker-assisted selection for provitamin A carotenoids. Our dissection of these two pathways supports future allele mining studies for development of nutrient dense maize.

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

P283

Identification and Validation of Maize Loci Controlling a Yield Component Trait via GWAS

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Advances in next generation sequencing (NGS) technologies and the development of appropriate populations and statistical methods enable the genome-wide dissection of the genetic determinants of traits. Several yield component traits were collected from four types of crosses associated with the Nested Association Mapping (NAM) population: NAM recombinant inbred lines (RILs, N=5,000), B73 x NAM RILs (N=700), Mo17 x NAM RILs (N=300) and a partial diallel of the NAM parents (N=250). A set of 4.9 million genic SNPs was identified via the analysis of RNA-seq data from four tissues from each of the NAM parents. These genic SNPs were combined with maize HapMap1 and HapMap2 SNPs to form a set of ~13 million SNPs that were projected onto the four cross type populations based on NAM parental and RIL genotypes. A joint composite QTL analysis was conducted using ~1,000 tagging SNPs from the Maize HapMap project. Next, three different approaches were used to conduct GWAS: 1) Bayesian-based simultaneous model fitting, 2) stepwise regression and 3) classical one-by-one SNP scanning. Approximately 20 trait associated SNPs (TASs) were identified by at least two methods. These SNPs, combined with an additional ~200 method-specific TASs were selected for validation using three unrelated populations. As compared to control SNPs, many of the TASs were associated with variation for yield component traits in one or more of three unrelated populations, thereby validating these TASs.

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P284

Identification of Sorghum Height and Maturity QTL in Nearly Isogenic BiparentaI Populations

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Sorghum is a photoperiod-sensitive, short-day tropical species that shows long delays in flowering at temperate latitudes. Most temperate-adapted sorghum cultivars are photoperiod-insensitive and dwarfed for grain production. Classical segregation studies predict that temperate adaptedness involves four major loci each for flowering time and dwarfing. Two major flowering loci, Ma1 (PRR37) and Ma3 (phytochrome B), and a single major dwarfing locus, Dw3 (PGP1/br2), have been cloned. Sorghum conversion (SC) lines are exotic varieties that have been introgressed with early flowering and dwarfing QTL from a common, temperate-adapted donor through backcrossing. To assess the phenotypic effects of individual introgressions from the temperate-adapted donor (BTx406), near-isogenic lines (NILs) were generated by crossing five diverse SC lines to their corresponding exotic pre-converted (PRE) progenitors. In summer 2012, F3 populations were grown to observe the phenotypic effects of the segregating introgressions, both alone and in combination. To fine-map the underlying uncloned loci, Illumina genotyping-by-sequencing was used to identify the BTx406 introgressions and recombinants in a 60 near-isogenic line sample from each of the five SCxPRE populations, mapped in 10kb intervals across the genome. Association mapping identified both the major cloned genes and several uncloned loci

P285

Identification of southern leaf blight resistance genes by genome wide association study

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Southern leaf blight (SLB), caused by the fungus Cochliobolus heterostrophus Drechsler, is one of the most damaging diseases worldwide in maize. A genome wide association study (GWAS) using the maize nested association mapping (NAM) population with 55 million SNPs from HapMap2 identified 1,296 loci statistically associated with SLB resistance. In a separate study, an association panel comprising 279 inbred lines was used to conduct a GWAS with 263,145 SNPs. Two independent analyses were performed for the association panel, based on the phenotypes obtained from five environments (BLUPs1) and three environments (BLUPs2) separately. A total of 277 SNPs (corresponding to 193 genes) and 363 SNPs (corresponding to 273 genes) significantly ($P<0.001$) associated with SLB resistance were identified from BLUPs1 and BLUPs2, while 62 genes are common between the two BLUPs. Nine candidate genes were identified in three of these analyses. Three of these genes localized within a small region of chromosome 3, and three in a small region of chromosome 4. Our findings provide interesting candidates for further research concerning the mechanism of resistance to SLB and plant diseases in general

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

P286

Inheritance of anthocyanin concentration in purple waxy corn (Zea mays L.) kernel and cob.

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The objectives of this study were to determine the inheritance of concentration of several antioxidant compounds in cobs and kernels from six crosses of waxy corn. For the cross KND 10-4P (P1) × BW (P2), F1, F2, BCP1 and BCP2 populations were developed. All genotypes were planted in a randomized complete block design with three replications at Khon Kaen, Thailand. The results showed that the average of cyanidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, total anthocyanin contents and total phenolic contents in cobs are higher than in kernels. Additive and epistatic gene effects explained the genetic variation for anthocyanin content of both kernels and cobs, but the additive gene action was the most important. Narrow-sense heritability estimates were moderate to high (0.51 to 0.88), indicating that these traits should respond well to selection. Implications for breeding waxy corn with improved anthocyanin levels are discussed.

Funding acknowledgement: United States Department of Agriculture (USDA), Thailand Research Fund

P287

Investigating the Maternal Genetics of Haploid Induction Rate

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Production of doubled haploid lines in maize can aid in accelerating breeding cycles allowing breeders to rapidly generate new inbreds for evaluation and testing. Production of maize doubled haploid lines is most commonly done *in vivo* using a paternal haploid inducer to pollinate a maternal donor population. One of the challenges associated with producing doubled haploid lines is the limited number of kernels which are successfully induced; usually around 8% depending on the inducer, the genetic background of the donor, and the environment in which the inductions were made. In an effort to improve this, genetic studies conducted by others have identified QTL controlling paternal induction ability. However, the genetic effect of the maternal donor is not fully understood. To address this question, 120 ex-pvp inbred lines were pollinated with the RWS/RWK-76 inducer hybrid in a two replication completely randomized design in the summer of 2012. Kernels were bulk harvested within each plot and haploid kernels were selected using the *R-nj* marker system. Haploid induction rates were then calculated by dividing the number of haploid kernels generated per plot by the total number of kernels harvested. Identification of low and high induction rates within the maternal donors will allow for the generation of a linkage mapping population that can be used to map QTL that control induction rate in maternal donors. This would allow breeders to focus their development of doubled haploids within genetic backgrounds that maximize the number of haploid kernels produced.

Funding acknowledgement: Raymond F. Baker Center for Plant Breeding

P288

Investigating the regulation of grain protein concentration in the Illinois Protein Strain Recombinant Inbreds: Precision phenotyping using *floury2*-mRFP as an alternative to NIR

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Maize protein is deficient in several essential amino acids due to their low proportion within the α-zein storage proteins that constitute the majority (50-60%) of total protein in the kernel. Reducing α-zein protein accumulation is an important goal of Quality Protein Maize (QPM) breeders worldwide. QTL mapping efforts suggest that grain protein is governed by many genes with small phenotypic effects, but genetic regulators still remain largely unknown. NIR has been used, but it is known to be influenced by environmental factors and measures an indirect phenotype. An alternative strategy described here is a precision phenotyping method that involves a red fluorescent protein reporter, mRFP, fused to the regulatory sequences from a single α-zein gene. We anticipate that the mRFP phenotype will allow for the separation of genetic factors that indirectly influence α-zein accumulation, such as those that alter starch synthesis due to the strong inverse relationship between endosperm starch and protein concentrations. Furthermore, because this method relies on direct imaging techniques for measuring mRFP, it does not require sample destruction. The mRFP transgene was crossed to the Illinois Protein Strain Recombinant Inbreds (IPSRIs), an advanced intermated RIL mapping population derived from the cross of Illinois High Protein (IHP) and Illinois Low Protein (ILP), and three years of phenotypic data have been collected on the IPSRIs using both NIR and mRFP methods. The results indicate that the mRFP method greatly reduces both environmental and within-sample variation when compared to NIR, resulting in higher heritability. Proof of concept will be reported for candidate genes involved in the zein synthesis and asparagine cycling pathways, which exhibit allele frequency shifts and expression variation in both the IPSRIs and the IHP and ILP populations. Ongoing studies include GWAS on the IPSRIs, where GBS SNPs will be associated with both NIR and mRFP phenotypic data.

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P289

Linear regression model to predict the agronomic performance of maize plants

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Actually different alternatives in breeding programs are being used around the world attempting to improve crop yield and nutritional value of new maize lines that can be able to grow in stressing environmental conditions such as lower temperatures. Metabolomics meaning can be understood as the characterization, identification, and quantification of molecules that are synthesized through biochemical processes in biological organisms. Advances in the development of metabolomics give us analytical platforms we use to investigate interesting metabolites, the quantity and nature, at different stages of plant growth. In this research we used VIS-UV spectrophotometry for carbohydrates determination. To evaluate some metabolites generated in corn grain we planted commercial varieties of white corn: Puma, Leopardo and Oso, and two varieties of yellow corn (2A120 and 2B150) with normal irrigation and proper fertilization of nitrogen under controlled conditions. During the flowering stage, maize ears became self-pollinated in order to obtain larger grains with improved nutritional qualities. At the senescence stage we registered some of the most known secondary traits such as stem weight and height, leaves number, tassel weigh and height and, corncob weigh. Using UV-VIS spectrophotometry we measured glucose, fructose, sucrose, starch and, total carotenoids content in maize grain. The metabolites highest level that we found are next: (2B150) Fructose, 23.59 mmol/L; (2B150) Glucose, 20.36 mmol/L; (Oso) Sucrose, 110.27 mmol/L; (Leopardo) starch, 102.00; (2B150) total carotenoids, 9.56 UA/gr dry weight. Tukey test shows genotype significant differences at 95% confidence intervals. Having the 200 weight of kernels from each variety as the response variable, a linear regression model was developed with Minitab 16 1.0 choosing “step-wise” forward option for finding the agronomic and biochemical predictors that deliver a reliable yield prediction. Statistical fitness linear model report shows that corncob weight and total grain weight variables perform as quadratic elements. The predictors that fit better the model are the secondary agronomic traits.

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P290

Mapping QTL controlling tolerance to Goss’s bacterial wilt of maize

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The re-emergence of Goss’s bacterial wilt and leaf blight, caused by *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*), poses a new challenge for maize breeders as tolerance is required of new hybrids, especially those bred for the western Corn Belt. Goss’s wilt symptoms include water-soaked lesions along leaf veins, which eventually lead to leaf blight and large reductions in grain yield. Severe infection early in development causes systemic infection, typically followed by plant wilting and death. Tolerance to Goss’s wilt appears to be a quantitative trait, with most genetic variation being additive. No experiments have been reported that attempted to map QTL controlling Goss’s wilt tolerance. The objective of this study was to further our understanding of the genetic basis of Goss’s wilt tolerance in maize using three of the 25 Nested Association Mapping (NAM) families:B73 x Oh43, B73 x HP301, B73 x P39. Artificial inoculations were made in the field during summer of 2012 and rated on an ordinal scale of 0-9. Additionally, a panel of inbred lines adapted to the North American Midwest was screened for Goss’s tolerance. We performed joint linkage composite interval mapping using the data from the three NAM families and genome-wide association mapping on the inbred panel using high-density marker data. Five QTL were identified that explain 30% of the phenotypic variation. Three QTL were common between two of the three NAM families screened. Although B73 is tolerant relative to the other parents, B73 carried an allele increasing susceptibility on chromosome 1 relative to Oh43 and HP301. Also, B73 carried an allele increasing susceptibility on chromosome 4 relative to P39. Results from this study will further our understanding of the genetic basis of variation for Goss’s wilt tolerance in maize.

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P291

Mapping QTLs with Maize-Teosinte NILs

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Various population structures have been utilized for QTL mapping in plants, such as F2, backcross (BC), doubled haploid, recombinant inbred lines, *etc*. Near isogenic lines (NILs) are often used to verify QTLs previously identified with other population structures. However, with efficient experimental design, NILs could be utilized for QTL detection and mapping. Furthermore, NILs reduce much of the "noise" caused by segregating genetic background, therefore providing more accurate allele effect estimates. Teosinte (*Zea mays* ssp. *parviglumis*) is the ancestor of cultivated maize and has more genetic diversity than inbred lines and landraces. Several studies have used the maize-teosinte F2 or BC1 populations to map QTLs related to domestication. To date, there is no similar research reported with maize-teosinte NILs. In this study, ten maize-teosinte introgression libraries comprised of 862 BC4S2 NILs were created and used to simultaneously detect, verify, and incorporated teosinte QTLs into an elite genetic background (B73). Kernel traits, including kernel row number and kernel size and shape, flowering time, plant height and ear height were characterized. An example of mining diverse alleles for a domestication trait will be presented to demonstrate one of the advantages for utility of the maize-teosinte NILs.

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P292

Mining for biofuel gold: Introgression mapping of maturity and height loci in sorghum.

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Sorghum, a tropical C4 grass, is an important crop worldwide with uses ranging from feed, both human and animal, to biofuels. Recent breeding programs, aimed at creating shorter, photoperiod insensitive plants, resulted in lines that were both high grain yielding and well suited for growth in temperate climates. This was achieved by the introgression of genomic regions from an elite donor line into the backgrounds of hundreds of diverse landraces creating Sorghum Converted (SC) lines. We utilized Genotyping-by-Sequencing to obtain dense marker coverage from 580 pairs of SC lines and their nearly-isogenic exotic progenitors, and to conduct a genome-wide analysis of the molecular changes controlling the height and flowering time traits. Mapping of introgression frequencies across all lines reveals three genomic regions involved in temperate climate adaptation, with multiple targets in each region. One of these regions, located on chromosome 6, coincides with a cloned dwarfing gene (Dw3) while the regions on chromosomes 7 and 9 coincide with previously identified QTL involved in both height and maturity. Several candidate genes for plant height/maturity variation identified in these regions could be exploited to improve biomass production in sorghum and related grasses.

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P293

Nonlinear effects of abiotic and biotic stress on yield and phyllosphere diversity.

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Plant leaves are inhabited by a diverse population of microorganisms that are important contributors to the overall health of the plant. We previously examined the relationship between bacterial diversity and pathogen resistance in Zea mays L. and it has been proposed that the presence of certain species of bacteria on corn leaves could increase resistance to fungal infection. It is important to look at how microbial diversity on corn plant leaves changes in relation to changes in the environment and how these changes contribute to the overall health of the plant. The purpose of this experiment is to examine how microbial communities change when exposed to multiple biotic and abiotic stresses.
Multiple IBM lines were planted and exposed to a combination of abiotic and biotic stress treatments: low nitrogen, drought and Southern Leaf Blight. Epiphyte samples were collected at week 7 after planting and species composition was determined using ARISA and fragment analysis. Measurements for cob diameter, plant height, and seed weight were collected and analyzed along with species composition to determine differences among treatment groups. These plant traits showed the expected decrease in the stress treatments with the presence of nonlinear effects. Disease ratings were taken and correlated with bacterial species diversity.

Funding acknowledgement: United States Department of Agriculture (USDA)

P294

Multi-environment validation experiments to assess the accuracy of phenotypic and genomewide selection within biparental doubled haploid breeding populations

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To our knowledge, no empirical studies have been published on the accuracy of genomewide selection across multiple years and locations. Our objective was to compare the accuracy of phenotypic selection and genomewide selection for different traits based on multi-environment validation experiments. Testcrosses of 150-250 doubled haploid (DH) lines from three elite biparental crosses were evaluated at six locations in 2008 or 2009. The 2008 and 2009 experiments, which corresponded to the first stage of testcross evaluation in a commercial breeding program served as the training data set. The testcrosses were evaluated at six locations in 2011 and six locations in 2012, and the 2011 and 2012 experiments served as the validation dataset. Two of the three crosses were evaluated in combination with two different testers in 2011 and 2012. All DH lines were genotyped with 3072 single nucleotide polymorphism (SNP) markers. We performed a cross-validation experiment to assess the effect of over fitting on the prediction ability of genomewide selection among untested individuals compared to the prediction ability among tested individuals in the training set. Correlations between genomewide predictions from the training dataset and observed performance in the validation dataset were moderate to high (0.30-0.70) for grain yield and high for grain moisture (0.54-0.75) for all three populations. Correlations between phenotypic values in the training dataset and validation dataset were moderate to high for all traits evaluated. The results from this study are encouraging for the routine implementation of genomewide selection in commercial breeding programs.

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P295

Numerical optimization of a marker-assisted backcrossing scheme for introgression library construction

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To study the genetics of environmental adaptation, we are developing a collection of near-isogenic lines with introgressions tiled across maize photoperiod QTL. While methods have been developed to backcross a single genomic interval from a donor parent into a recurrent parent genetic background, methods to produce an introgression tiling path are not available. We propose an algorithm for marker-assisted backcrossing that uses marker data, linkage map and pedigree information to optimize the construction of tiling paths by finding the set of individuals maximally representative of a defined ideotype set of isogenic lines. In backcross generations, optimization is based on minimizing distance of recombination events from the ideotype boundaries and maximizing recovery of recurrent parent genome in the non-targeted region of the chromosome. In addition, in selfing generations, homozygosity is maximized.

Using simulations, we test whether or not the algorithm selects optimized sets of individuals when available in successive generations. In this study, tiling path construction across four distinct regions of the maize (*Zea mays* L.) genome was investigated, using the nested association mapping linkage map as a basis for the simulation parameters. Assuming a count-location model for recombination, we compute the number of families and individuals per family that would need to be genotyped over successive generations to produce an optimal set of isogenic lines constituting a desired introgression library. Finally, we discuss how varying the number of families and individuals per family affect the quality of the tiling path.

Funding acknowledgement: United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA)

P296

Optimal resource allocation for a maize genomic recurrent selection program

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The allocation of resources between population size and replication is a critical issue in the design of phenotypic and marker-assisted selection (MAS) programs. Because alleles are replicated across individuals in QTL mapping and MAS, it has been recommended that more resources be allocated to increasing population size at the expense of replication. Genomic selection is a form of MAS using all marker information simultaneously to predict individual genetic values for complex traits and has widely been found superior to MAS in terms of selection accuracy. Studies using a simulated biparental population previously showed that response of prediction accuracy to resource allocation strategies differed between genomic selection models (RR-BLUP) and marker-assisted selection models using ordinary least squares estimation (OLS), leading to different optimal resource allocation choices. For OLS, it was always advantageous to maximize population size at the expense of replication, but a higher degree of flexibility was observed for RR-BLUP. We've extended these findings to include an analysis on resource allocation for genomic selection within a maize synthetic population undergoing recurrent selection. Properties of synthetic populations that will affect findings on resource allocation for genomic selection include variable minor allele frequencies at QTL and marker loci (in contrast to 0.50 for biparental populations), segregation of more than two alleles, and faster decay of linkage disequilibrium. Our results will assist maize breeders practicing phenotypic recurrent selection transition to genomic recurrent selection as genotyping costs continue to decline.

Funding acknowledgement: United States Department of Agriculture (USDA)

P297

Optimizing sampling for estimation of genetic architecture and prediction of phenotypes

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Phenomics and high-throughput sequencing have increased the information available to geneticists. From this information, linkage and association mapping of alleles and genomic prediction of phenotypes has enhanced our knowledge of genetic architecture. Given these advances, we are left with several questions: (1) How many genotypes shall we sequence to sufficiently understand a population's allele frequencies and linkage disequilibrium? To predict phenotypes? (2) Can we incorporate past knowledge of phenotype, pedigree, or genetic architecture to reduce this number relative to an uninformed approach? (3) At the expense of resolution, can we obtain a robust coarse-grained estimate of genetic architecture to predict a population's response to selection and inform mating decisions? To address these questions, we developed sampling criteria for individuals when sequencing a populations in which no prior knowledge of phenotype or relatedness exists, as well as those with prior knowledge of the attributes. We also began exploring methods to coarse-grain the genome. These seek to balance the trade-offs between bias and variance in marker assisted selection and genomic
prediction.

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P298

Phenotypic and Genetic Dissection of Maize Internode Length

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Plant height is among the most studied traits in maize, and is a function of two component traits, internode length and node number which is associated with flowering time. With the growing interest in utilizing maize as a source of biomass for biofuel production, it is desirable to alter the maize ideotype to enhance not only grain yield but also biomass yield without altering maturity. This could be achieved through the development of plants with superior plant height. The objectives of this study were to characterize the phenotypic diversity, determine the relationships among plant height and internode length, and map quantitative trait loci (QTL) for these traits using two recombinant inbred line populations and two association panels. Our results indicate that plant height can be phenotypically and genetically dissected into node number and internode length, but variation in lengths of individual internodes makes internode length less heritable than node number. Internode length below the uppermost ear showed a strong association with plant height and internode length, but was weakly correlated with node number and flowering time. Plant height, node number, and flowering time were moderately correlated to each other. Several QTL and single nucleotide polymorphisms were associated with internode length below the uppermost ear. QTL-hotspots were identified for plant height, internode length, and internode length below the uppermost ear. This study constitutes an important step towards the characterization of the genetic architecture of internode length, a key trait in maize breeding and genetics.

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P299

Phenotypic Response to A. avenae Infection in the IBM 94 Maize Population

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Corn is cultivated on 79 million acres in the US, and its value is in excess of $50B. Not only is corn an essential world crop and ideal model organism, but it is currently being employed in pharmaceuticals to synthesize enzymes that can be used to treat lysosomal storage disorders. Acidovorax avenae subsp. avenae (Aaa) is a bacterial pathogen that infects maize, causing bacterial wilt. Shredding infected leaves and causing leaf streaks, bacterial wilt can cause serious crop damage, especially in sweet corn. There exists variation in the susceptibility of maize genotypes to Aaa. The object of this study was to identify the loci responsible for resistance through inoculation of 95 recombinant inbred lines (RILs) of the intermated B73 and Mo17 (IBM) maize population, and to quantify their subsequent reactions. The reactions were evaluated phenotypically on a scale of 0-100% area infected, and was plotted against the genotypic value given using a whole genome scan. This analysis was performed in QTL (quantitative trait loci) Network which identifies the loci responsible for Aaa resistance. These results may then be used to breed lines of maize with enhanced Aaa resistance, thus limiting disease and improving crop yield. This data will also contribute to existing databases for this population, furthering research and information availability in maize resistance.

P300

Plant genetic contributions to microbial colonization in the rhizospere and roots of several panicoid grasses

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The microbial community composition and dynamics in the rhizosphere is influenced by the host plant and by a variety of other environmental factors. Determination of the influence of host genetics on the composition of the rhizosphere microbiome may be useful in understanding how plants mobilize micronutrients and avoid parasitism, especially in the earliest stages of seedling development. Towards this goal, we studied the plant genotype dependence of microbial colonization by rRNA gene sequencing of metagenomic samples from the rhizosphere and roots of switchgrass, Setaria species and maize. Our results suggest considerable difference between rhizosphere and root bacterial communities (in each genotype) as well as genotype-dependent colonization of root-associated microbes. For example, in switchgrass, we found that the upland cultivar (summer) and the lowland cultivar (Alamo) exhibited differences in root colonization for microbial phylotypes such as Bradirhizobium, Devosia, Niastella, Glomus and Gigaspora. Setaria species showed genotype-dependent colonization for Azospirellum, Ideonella, Ralstonia and Steroidobactar. Avalability of a genetic map, genome sequence and mapping populations for Setaria (S. italica x S. viridis) presents an opportunity to map QTLs that are associated with differential accumulation of interesting microbial phylotypes.

Funding acknowledgement: Department of Energy (DOE)

P301

Production of biofuel from cellulosic biomass

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Increasing world population and limited resources is necessarily synonymous with energy crisis. Fossil fuel has been exploited since industrial revolution and have contributed to polluting the earth to nearlethal limits. In this situation fuel from biological organisms (Biofuel) is very promising in meeting the global energy demands and also restoring balance in the ecosystem. It is renewable and clean sourceof energy. Biofuel though produced from various sources is not yet commercially viable due to its high cost in production. In our lab we are focusing on cellulosic biofuel production from sorghum stalks.
Sorghum is a very promising energy crop as it can grow in relatively hostile conditions with low input in terms of fertilizer and water, also has a high biomass (measured by its height and maturity). This unique character of sorghum can silence the bioethical issues that are inherent to biofuel production as it can be grown on land considered marginal for growing food crops. High biomass along with high saccharification yield (conversion of cellulosic biomass to fermentable sugars like xylose and glucose) will ensure a more cost effective biofuel. To improve saccharification yield, our lab is trying to identify candidate genes especially involved in cell wall synthesis that affects the process of saccharification.Genotyping and association mapping are in progress using genetic markers with bioinformatics tools like TASSEL and GAPIT.

Funding acknowledgement: University of Louisiana at Lafayette

P302

QTL mapping for P efficiency and root traits under low phosphorus availability in maize and identification of putative PSTOL1 homologues

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Phosphorus (P) is one of the most limiting mineral nutrient for plants and crucial to the productivity of several crops, including maize. However, the low P is a primary constrain for maize productivity. One strategy adopted by plants under P starvation is the modification of their root morphology. In rice, a QTL (Pup1) was associated with P efficiency and increased root growth under low P. Recently, a gene underlying Pup1 locus was identified and denominated Phosphorus-starvation tolerance 1 (PSTOL1). This gene enhances root growth when overexpressed, improving P uptake and consequently grain yield. In maize, several QTLs for root traits were identified under P contrasting levels in nutrient solution and field conditions. Nevertheless, the relationship between root architecture and P efficiency remain unknown and candidate genes to these traits have not been identified yet. Thus, this work aimed to analyze the relationship between root morphology and P efficiency as well as the genetic basis underlying these traits in maize. We performed QTL analyzes to search for a functional homologue of OsPSTOL1. Based on rice PSTOL1 amino acid sequence we searched several maize sequence databases. We selected 12 predicted proteins with more than 50% identity with rice and Arabidopsis protein kinases. These proteins clustered in three major subfamilies. We found four predicted genes with more than 65% identity with OsPSTOL1, which were located on chromosomes 3, 4 and 8. We identified three to four QTLs for each trait, explaining 23.5 to 45.3% of the total genotypic variance. The QTLs for the analyzed phenotypic traits were concentrated in few genomic regions, mainly on chromosomes 1, 8 and 10. We observed the co-localization of Zm1_PSTOL1 with QTLs clusters, including root morphology traits, total plant dry weight and P acquisition efficiency, on chromosome 8. We also observed that Zm1_PSTOL1 expression occurred only in the parental line that was the donor for these QTLs. These results represent the first evidence that the gene Zm1_PSTOL1 can be related to P acquisition efficiency. However, additional studies are needed to validate this gene as Pup1 functional homologue in maize.

Funding acknowledgement: FAPEMIG, CAPES, CNPq, Embrapa,GCP

P303

QTL mapping of resistance to Aspergillus flavus infection in maize (Zea mays L.)

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Cereal crop maize is subject to be infected by pathogen Aspergillus flavus (Link: fr), resulting in aflatoxins production which poses a great threat to people, poultry, and animals health. Herein, we report QTL mapping of resistance to A. flavus infection in maize. A susceptible inbred line M5P and a resistant inbred line RA were selected from diverse maize germplasm and adopted to develop recombinant inbred line (RIL) populations for QTL mapping. A total of 122 polymorphic SSR markers were evenly distributed on maize 10 chromosomes and spanned 1,511.6 cM in length with an average distance of 12.39 cM per marker. Three QTL contributing resistance to A. flavus infection were detected on chromosomes 1, 4, and 7 in F6 population. Two resistant QTL were detected on chromosomes 7 and 8 using F7 population. Identified QTL could account for 4.89—17.46 % of the total phenotypic variation. Of note, QTL on chromosome 7, which was detected in both F6 and F7 populations, could be served an important target for the following fine-mapping and breeding programs with the goal of relieving A. flavus infection in maize production.

Funding acknowledgement: National Basic Research Program (2009CB118400)

P304

Reciprocal Differences in the Expression of *Corngrass1* (*Cg1*).

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Corngrass1 (*Cg1*) affects the transition from the juvenile to adult phase of vegetative growth. Adult *Cg1* plants maintain characteristics of juvenile plants including the presence of epicuticular wax throughout the entire plant, excessive tillering, and reproductive structures consisting of vegetative tissues. The severity of morphological expression of *Cg1* varies continuously indicating that other factors affect vegetative phase change. The objectives of this experiment were to determine if inbred background (B73, Mo17) and/or parental (male or female) source of *Cg1* affected the severity of expression of the *Cg1* phenotype. We developed six B73 Cg1 near-isogenic lines and six Mo17 Cg1 near-isogenic lines. These lines were crossed reciprocally with wild type B73 or Mo17, resulting in a total of twenty-four *Cg1* hybrids. Trials were grown in replicated trials at four locations. Data were recorded for plant rating, tassel rating, ear height, tiller number, ear number, top leaf width, and ear leaf width. Hybrids with *Cg1* as the male parent consistently expressed the *Cg1* phenotype more severely than hybrids with *Cg1* as the female parent. Hybrids with *Cg1* male parents were significantly different from those with *Cg1* female parents for plant rating, tassel rating, tiller number, juvenile wax, ear height, and ear leaf width. Inbred background (B73 vs. Mo17) did not have a consistent effect on the expression of *Cg1*.

Funding acknowledgement: University of Wisconsin-Madison College of Agricultural and Life Sciences

P305

Resistance to barley yellow dwarf virus in segregating populations of maize

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With increasing winter temperatures in Germany, barley yellow dwarf virus (BYDV) is expected to become a prominent problem in maize cultivation. Breeding for resistance is the best alternative to control the disease and break the life cycle of the virus. The objectives of our study were (I) to determine phenotypic and genotypic variation in five segregating populations of maize with respect to BVDV tolerance and resistance and (II) to quantify the influence of BYDV infection on plant performance traits. In 2011, five segregating populations with a total of 445 genotypes were grown at two locations in Germany as well as in greenhouse experiments. Plants were inoculated with the virus BYDV-PAV transmitted by aphids of the species *Rhopalusiphum padi*. We observed considerable genotypic variance for the traits virus content as measured by ELISA as well as symptom occurrence. Furthermore, heritabilities were high for the plant performance traits, ear height and plant height. Correlation coefficients between the pairs of traits were significantly different from 0 (α=0.05) but low. Inoculated plants were reduced in plant height compared to not inoculated plants. The results of our study suggested a high potential for breeding of BVDY resistant maize.

Funding acknowledgement: Federal Ministry of Food Agriculture and Consumer Protection (BMELV)

P306

Root morphological analysis of a maize diversity panel under low and high phosphorus

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Phosphorus (P) is an essential macronutrient for plants, which is acquired from the rhizosphere solution as phosphate (Pi). The concentration of Pi in the soil solution is often low, therefore the supply of Pi to the root surface by diffusion is slow. Hence, P is one of the least available mineral elements in the soil and frequently limits plant growth. The modular structure of roots enables them to quickly respond to their surrounding environment, making plants more adaptable to environmental changes. Certain root system types can help increase the yield due to their higher capacity to acquire Pi. Our work aimed to explore the diversity of root morphology related with P acquisition efficiency. We used a paper pouch system with Magnavaca´s nutrient solution (2.5 and 250 uM P) under a controlled environment. We evaluated four root traits (length, volume, diameter and volume of fine roots), dry weight and P content of a maize diversity panel, composed of two hundred inbred lines from Embrapa Maize and Sorghum breeding program. Low coefficient of variation and high heritability were detected for all analyzed traits. Significant differences for genotypes and P dose were detected for all traits. The interaction between genotype and P was significant for all traits except, root average diameter, total dry weight and shoot dry weight. A correlation among root traits and dry weight was observed in both low and high P conditions. Frequency distribution and Principal Component Analysis enabled us to observe a great diversity of root system types within the population in both conditions. Genotypic variation for root system has been associated with substantial variation in the acquisition of P. The utilization of root traits in crop breeding program would be greatly facilitated by a better understanding of the genetic, physiological and environmental regulation of root system elements.

Funding acknowledgement: FAPEMIG, CAPES, CNPq, Embrapa,GCP

P307

Root morphology comparison between maize recombinant inbred lines population per se and crossed with a common tester under low phosphorus condition

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Phosphorus (P) is highly immobile in soil, therefore difficult to be acquired by plants. Low P is one of main constrains to increase maize yield in Brazil. Root traits that enhance topsoil foraging are important for P acquisition and since they are controlled by multiple genes, one useful tool for this analysis is recombinant inbred lines (RILs). Most studies regarding root traits have been done in RILs per se, but for breeders it is important to know how lines perform in crosses. We know that heterosis is of paramount importance in maize breeding and is manifested during the early stages of root development. Hence, we phenotyped a population of 145 RILs, derived from a cross between maize contrasting lines for P efficiency (L3 – efficient and L22 – inefficient), and the same RIL population crossed with a common tester (L53 - inefficient), for root traits. We used a paper pouch system with Magnavaca´s nutrient solution (low P - 2.5 uM) under a controlled environment to evaluate four root traits (length, volume, diameter and volume of fine roots) and shoot and root dry weight. High heritability and low coefficient of variation were detected for all analyzed traits for both populations after 13 days of treatment. As expected, the hybrids showed higher values for all traits. Interestingly, all traits correlated significantly between RILs per se and crossed. Based on the correlation between root traits and dry weight, we could observe that plant investment in root turned into shoot biomass. Thus the metabolic cost seems to be advantageous in this case. Additionally, Principal Component Analysis (PCA) enabled us to differentiate contrasting maize populations based on the selected traits. PC1 had positive eigenvector coefficients for all variables, except for root diameter. PC1 was explained most by all traits, except root diameter and PC2 was explained mostly by root diameter. These phenotypic results will be used in the discovery of root morphology quantitative trait loci (QTLs) that are also involved on P acquisition efficiency in maize and also help to develop cultivars that use less fertilizer.

Funding acknowledgement: FAPEMIG, CAPES, CNPq, Embrapa,GCP

P308

Screening maize (Zea mays L.) germplasm for the crtRB1 and LcyE polymorphisms to increase β- carotene content in Indian conditions..

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According to World Health Organization Vitamin A deficiency causes blindness in 250,000 to 500,000 children per year and death in 670,000 children per year. In order to address this global problem the biofortification of maize is considered to be a cost effective and sustainable approach. Maize is a widely consumed food crop that displays considerable natural variation for vitamin A precursors namely, α-carotene, β-carotene and β-cryptoxanthin. Selection of maize inbreds for increased β-carotene will enable plant breeders to develop locally adapted maize varieties and hybrids with improved β-carotene content. Polymorphisms in LcyE and crtRB1 region in the maize genome are associated with increased β-carotene content in maize. In this study 210 maize inbreds were obtained from CIMMYT (International Maize and Wheat Research Centre) and DMR (Directorate of Maize Research) and their morphological characters were evaluated. The maize inbreds that displayed highly desired morphological characters are CIMEntry94 (Ear Length), DMWNY4017 (No of grain rows) and DMWNY4019 (Early). These inbreds can be used as donors for desirable morphological traits in back cross breeding programs. The inbreds CIM Entry94 (Ear Length), DMWNY4017 (grain rows) and DMWNY4019 (Early anthesis and silking, 51 and 56 days respectively) possessed the desirable traits for superior hybrid synthesis. All the 210 maize inbreds were screened for the presence of LcyE3'Indel region of LcyE gene and HYDB 3'TE region of crtRB1 gene. Inbreds UMI176, DMWNY4091, DMW NY4042, DMWHOY4304, DMW SCY4397. DMWHOY4324, DMR113, DMWNY4058 and CIMEntry68 had the crtRB1 polymorphism. It was found that the inbreds DMWNY4042, DMWNY4055, CIM Entry28 and CIM Entry112 had 8 bp deletion at 3' end of LcyE gene. HPLC studies were done to confirm whether these inbreds had high β-carotene content.

Funding acknowledgement: Department of Biotechnology - India, Tamil Nadu Agricultural University - India.

P309

Stepwise regression for grain yield of maize hybrids under drip irrigation

(submitted by Soheil Zarandy <s_zarandy@yahoo.com>)
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In experiment in 2012 in farm of research fadak agro-industry company of Qom province had performed. Experimental design was a randomized complete block with three replications under drip irrigation. And 9 hybrids studied water with electric direction (EC) 2 desi zimense and water's pH was equal with 7.4 and tds=1400. Irrigation interval was 4 days and used was the plastic hose for the irrigation.In implant time in every row 60 heap had been sawn that in every heap implant 3 seed.Distance of cultivation rows was 75 centimeter and distance of heaps were 25 centimeter implant line length was 6 meter.
At first phase, by using of many variables regression with stepwise method, corn grain yield average as accessory variable and another characters as independent variable had been checked and resume of their result had been write in tables. Obtained result showed that, earlefa area , in soreness shape, explanted 26.39 present and escort with bush height, seedrow, to ear height leaf, flag leaf area 97.04 present of corn grain production changes. If grain yield = Y and earlefa area characters = x1 , bush height = x2 , seedrow = x3, to ear height leaf = x4 , flag leaf area = x5 → general equation as stepwise is beneath shape:
Y = -38271 - 78 X 1 + 106 X 2 + 1700 X 3+ 38 7X 4 + 62 X5
This equate show that earlefa area effect more than another characters on grain yield production and if are can rising earlefa area grain yield increase.Name hybrids:KSC500,KSC302,KSC320,KSC301,G-54190,G-54193,G-3261,OSSK444.

P310

Study correlation analysis between characters in maize hybrids in normal condition

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In order to study the reaction of maize hybrids to salinity condition , an experimental was conducted at experimental field station of Agricultural and Natural Resource Research center of Qom province under Two conditions (normal and salinity stress)during the cropping season of 2006-2007.Experimental design was a randomized complete block with three replications.Soil tissue with electrical conductance (EC) in depth of 0-30 was 8.5dissilience and in depth of 30-60,was 9.9dissilience and the soil pH was equal with7.6 .
The character of seed number in a row with corn diagonal in possibility level have0.1meaningful correlation,it means that with increasing in row,the corn diagonal will increase.Also,the correlation low number character with corn diagonal in possibility level has0.1meaningful (0.51**)and if the number of ow was more, corn diagonal will be further.The correlation of seed number character in row with corn length in possibility level is0.1meaningful.It means that with increasing of seed number in arrow,the corn length will be increase.The character of row number in possibility level has0.5negative correlation with corn length. The characters of corn length and number of seed in row with character of corn weight,respectively have0.1and0.5meaningful correlation in possibility levels.It means that if the corn length increased, the corn weight increase,too.And if the number of seed in arrow increased,the corn weight increase,too.The character of corn number in bush with weight of thousand seed in possibility level has0.5negative meaningful correlation.
Name hybrids(27):ZP 434, KSC 500, KSC 340, KSC 400, KSC 302, KSC 260, KSC 320, KSC 250, KSC 301, G- 54190, G- 54193, G- 54186, G- 54185, G- 3337, G- 3261, OSSK 444, BC 404, BC 354, OSSK 373, BC 282, KSC 350, BC 572, BC 418, BC 504, ZP 341, NS 540, OSSK 499.

P311

Study correlation analysis between characters in maize hybrids under drip irrigation

(submitted by Soheil Zarandy <s_zarandy@yahoo.com>)
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In experiment in 2012 in farm of research fadak agro-industry company of Qom province had performed. Experimental design was a randomized complete block with three replications under drip irrigation. And 9 hybrids studied water with electric direction (EC) 2 desi zimense and water's pH was equal with 7.4 and tds=1400. Irrigation interval was 4 days and used was the plastic hose for the irrigation. In implant time in every row 60 heap had been sawn that in every heap implant 3 seed.Distance of cultivation rows was 75 centimeter and distance of heaps were 25 centimeter implant line length was 6 meter.
The character of row with corn Ear diameter in possibility level have %1 meaningful correlation, it means that with increasing in row, the corn diagonal will increase.The character of ear leaf lenght with corn Ear diameter in possibility level have %5 meaningful correlation.The character of flag leaf width with corn flag leaf area in possibility level have %1 meaningful correlation,it means that with increasing flag leaf width, the flag leaf area will increase.The character of row with corn Ear diameter in possibility level have %1 meaningful correlation, it means that with increasing in row, the corn diagonal will increase.The character of ear leaf lenght with corn Ear diameter in possibility level have %5 meaningful correlation.The character of flag leaf width with corn flag leaf area in possibility level have %1 meaningful correlation , it means that with increasing flag leaf width, the flag leaf area will increase.Name hybrids:KSC500,KSC302,KSC320,KSC301,G-54190,G-54193,G-3261,OSSK444.

P312

Study on effects of drought stress and mycorrhizal fungi on water use efficiency in corn silage

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Dehydration is one of the most important factors limiting the proliferation of products in arid and semiarid areas and growth reduction due to drought stress is far more than any other environmental stress. Iran is located in arid and semi-arid climates of the earth and two important environmental factors such as drought and salinity are the parameters limiting plant growth in these areas. Considering the recent drought, the intense drop in underground water in recent years and severe shortage of water in agriculture sector in Iran, the use of bio-fertilizers seems inevitable. Mycorrhizal fungi have symbiosis with most plants and improve their growth. By colonization of host plant roots, the resistance to drought stress in these plants increases and increased resistance to drought, results in changes in leaf, improving leaf water, Turgescence potential and keeping the stomata open. In drought stress, these fungi can provide benefits for plant by improving the absorption of phosphorus and water. other benefits of these fungi can also be increasing water use efficiency (WUE), increasing concentrations of phytohormones, and increasing plant resistance to heavy metals.

Maximum water use efficiency, 8.9 kg per cubic meter, belongs to Single Cross 704 and the minimum amount of it, 4.174 kg per cubic meter, belongs to Double Cross 370 without bio-fertilizer without mycorrhiza. The reason for increased water use efficiency under water stress conditions is that plants lose their extra leaves in these conditions, rduce their leaves area and hold their stomata closed or semi open to reduce water loss through evaporation and transpiration. As a result, the plant uses the water to produced dry seeds and this results in increased water use efficiency. Also, the main reasons for the increase in WUE in mycorrhizal plants is increased hydraulic conductivity in roots of mycorrhizal plants. So water will be transferred with higher efficiency and the mycorrhizal plants produce more root biomass. So, the nutrient uptake and efficiency of water transport and photosynthesis in these plants increases. Mycorrhizal roots have different physiological and biochemical properties which can be effective in increasing the uptake. They can acidize rhizosphere by increasing proton seeps or increasing co_2 pressure which can mobilize phosphorus in calcareous and neutral soils. Also, Mycorrhizal produces phosphatase enzymes which can phosphorus mobile and absorbable from its organic resources. We can say that the main role of mycorrhizal is to supply phosphorus for the plant roots. The highest amount of phosphorus, 1.45 grams, belongs to single cross 704 and the lowest amount, 0.17 grams, belongs to maxima. Drought stress caused a significant increase in the amount of protein in drought conditions. The highest amount od protein, 20.6% belongs to double cross 370 using mycorrhiza with stress on the first level (BBCH 51), and the lowest amount, 8.125% belongs to single cross 704 (normal) without stress. This can happen because of the increase in amounts if amino acids and proline in drought stress conditions which can finally lead to an increase in amount of protein in plant.

By use of three varieties of corn silage, single cross 704 (normal), double cross 370 and maxima with two types of mycorrhizal fungi Glomus moseae and Glomus faciculatum with two levels of drought stress in BBCH 51 and BBCH 61, it was found that water use efficiency in normal type (704) was higher in comparison with the other two types. The percentage of phosphorus in single cross 704 (normal) in comparison with the other two types has had a meaningful effect. This was because of the effects of mycorrhizal in this study. It was also observed that the amount of protein in plant has considerably increased in stress condition.

Keywords: stress, Mycorrhizal fungi, protein, water use efficiency, phosphorus, corn silage

P313

The impact of recombination on allelic expression in the *Bz1/Sh1* Interval of Zea mays

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Genetic diversity is generated through sequence variation that arises from alterations in an organism's genetic code. One naturally occurring source of sequence variation is meiotic recombination. The frequency and distribution of recombination is significantly variable across the genome, partitioned into recombination hotspots and coldspots, which are chromosomal sections that display either increased or decreased rates of recombination. It has been hypothesized that areas of recombination experience elevated mutation rates due to chromosomal rearrangements and inaccurate break repair, and that these regions may experience more rapid fixation of alternate alleles in populations. In an effort to understand the influence of recombination on adjacent regions of the genome, we have focused on the *Bz1/Sh1* region of maize, a well-known recombination hotspot. The maize inbred lines W22 and B73 exhibit extensive sequence variation in the intergenic across the *Bz1/Sh1* interval, while maintaining sequence conservation and colinearity of genes in this region. Analysis of allelic expression in this region indicates significant expression variation for genes adjacent to previously identified recombination hotspots from maize hybrids of various genotypes, although recombination frequencies among W22/B73 lines for this region have not been described. Specifically, major expression differences are observed for sesquiterpene cyclase (*stc1*) and *tac6058*, a gene of unknown function. Here, recombinant individuals were produced by self-pollination of W22 x B73 hybrids. Next, a PCR based method was developed to exploit sizeable indels for delineating the location of recombination breakpoints. Breakpoints have been determined for 82 recombinants, and preliminary results show a recombination hotspot near the significantly differentially expressed genes.

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P314

The molecular characterization of a MAGIC population reveals high potential for gene discovery

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LD mapping has become a method of choice to identify genomic regions involved in the variation of both qualitative and quantitative traits in many species. In maize, the wide genetic diversity is highly structured among genetic origins and hetetoric groups, and panels of lines from a broad genetic diversity thus exhibit the same strong structure. This structure is unfortunately detrimental to the identification of true positive associations (increases type I and II error risks) even if statistical models devised to control the effect of the structure are used. To overcome this limitation, BIOGEMMA has undertaken the development of a Multiparent Advanced-Generation Inter-Cross (MAGIC) population crossing 16 historical lines of the representatives the most significant heterotic groups used for hybrid production in temperate regions. The 10th generation of intercrossing was completed during the Winter 2011-12. At the 3rd generation of intercrossing, 543 MAGIC lines were extracted by haplo-diploidization. MAGIC lines and founder lines were genotyped using the Illumina MaizeSNP50 beadchip. The molecular characterization of a panel of MAGIC reveals promising features for fine-mapping genetic factors involved in the agronomic traits: large size of the panel (≈ 400 lines), large genetic diversity, absence of internal genetic structure, relatively low LD especially near the telomeres, high statistical power. Moreover, the inference of parental alleles in the HD lines has been efficient and has provided an original information for GWAS. BIOGEMMA is currently genotyping the panel with 400.000 SNPs and testcross progenies of the HD lines have been phenotyping in different environments since 2011. The panel of MAGIC lines developed by BIOGEMMA is an attractive resource to ease the identification of good candidate genes for developing genetically modified traits and also to provide informative molecular markers usable in Marker Assisted Selection (MAS) or genomic selection programs.

P315

Towards the fine mapping of a major QTL controlling the number of rows of kernels per ear

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In the process of crop domestication, intense artificial selection of morphological traits created an ideal scenario to study the evolution of morphologic divergence. In maize (*Zea mays* ssp. *mays*), selection during domestication increased the kernel row number from two in its wild ancestor (*Zea mays* ssp. *parviglumis*) to more than 10 rowed cobs in modern maize. Kernel row number is a polygenic trait that has drawn attention due to its agricultural importance in grain yield. A study in our lab identified QTLs for kernel row number in all ten chromosomes of maize, with one of the highest LOD-score QTLs located in the long arm of Chromosome 1 (LOD 56.91). In the present study, we are using progeny lines segregating for this QTL region, which were derived from a cross between a midwest maize inbred (W22) and teosinte, backcrossed twice to maize and self pollinated for three additional generations (BC_2 S_3) resulting in recombinant lines carrying teosinte introgressions. Our goal is to narrow down the original QTL 1.5 LOD support interval of 273 kb in chromosome 1L and to find the causative gene(s) responsible for kernel row number.

Funding acknowledgement: National Science Foundation (NSF)

P316

Using GBS data to study the distribution of recombination breakpoints in two maize NAM populations

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Low coverage GBS (Genotyping by Sequencing) data provides genotypes at more than one million sites across the maize genome. For that reason, it has the potential to provide high resolution estimates of the position of recombination breakpoints. On the other hand, a high proportion of missing data and mis-calling of heterzygotes both present challenges for using GBS data for this application. Miscalling of heterzygotes occurs when only one of the potential alleles is sampled for an individual. We describe methods using a hidden Markov model to overcome these problems and use GBS data to study the distribution of recombination breakpoints in two maize NAM (nested association populations). The first population was created in the US by crossing 25 diverse inbred lines to a common parent (B73) and selfing each for several generations to create 200 RILs per population. The second population was created in China by crossing 11 inbred lines to a common parent (Huangzaosi) and creating RILs from those. We show that the overall distribution of breakpoints is very similar regardless of the lines crossed but that some significant differences do exist. We also examine overall differences between the B73 populations and the Huangzaosi populations, where recombinations in over 7000 lines provide substantial power. Biological implications of the observed differences are discussed.

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P317

Using NAM to put a dent in our understanding of maize kernel type

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Much of our modern maize germplasm was originally derived from the combination of northern flint lines and southern dent lines. Yet commercial production in the US today is dominated by dent or semi-dent kernel type corn (Corn Belt dent), which has hard outer walls of endosperm surrounding a soft floury interior that, when dried, compacts to form the characteristic dent in the top of the kernel. One major exception is flint type corn, which is grown in some colder, short season areas of North America, Europe, South America, and widely in tropical and developing areas. Flint corn is characterized by its rounded, vitreous outer endosperm and soft granular center and has desirable qualities such as cold tolerance, disease and insect resistance, as well as longer storage capacity than many dent lines.

The Nested Association Mapping (NAM) population parent inbred lines represent many of the major kernel types found in maize. In 2006 the entire NAM population was grown and scored visually for kernel type in five locations. The NAM population contains nine flint and nine semi-dent parent lines with a common dent parent, B73. Linkage mapping was used within and across families, and ~7,400 intervals, to look at flint by dent, and semi-dent by dent crossed families of NAM. Areas of the genome that are significantly associated with the difference in kernel type seen in NAM were successfully located. Seven of the nine flint families, as well as half of the semi-dent families, in NAM share one major QTL on Chromosome 4. Several other major and minor QTL are also shared across many families. Major QTL and candidate genes that characterize the diversity in the flint kernel type in the NAM population will be shown.

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P318

Using QTL Enrichment to Improve Nitrogen Utilization in Maize

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Increased nitrogen use efficiency (NUE) is an important target for future maize improvement. Essential to the design of an effective breeding program to select maize hybrids with enhanced NUE is an understanding of past progress, variation among maize germplasm for NUE and its component traits, and identification of phenotyping approaches to optimize genetic gain. We documented genetic variation for NUE and its component agronomic traits among a diverse collection of historical and recent elite maize inbreds and hybrids grown in field trials with different levels of soil N supply. Many of the genotypes evaluated also represent important resources for maize functional genomics. The results confirm previously reported trends for modern elite compared to historical hybrids, where grain yields have increased as a result of superior tolerance to higher plant densities, greater harvest index, and reductions in grain protein concentration. In addition, we demonstrate that past breeding has likely optimized N uptake for high grain yields, but that significant opportunities exist to further improve how maize plants utilize acquired N. We developed a phenotyping approach that estimates N utilization as the ratio of total biomass relative to total plant N, which effectively controls for the significant impacts of N-level, relative maturity, and heterosis on this trait. Using this measure of total N utilization, we identified the allelic genotypes associated with enhanced N utilization in the IBM population at nine previously identified potential NUE Quantitative Trait Loci (QTL). Selection of IBM lines with haplotypes showing maximum enrichment of high NUE associated alleles at the potential QTL shifted the population mean for NUE. Coupled with lines selected for minimum enrichment at the same locations, these enriched lines capture the diversity in the original population with minimal field space and phenotyping providing a new strategy for future trait assessment.

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P319

A reverse screen to identify maize genes involved in the response to phosphorus starvation

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Phosphorus (P) is one of the most important elements for plant growth and development, and yet availability in the soil is frequently limited, and, in consequence, large quantities of P fertilizers are required in agricultural systems. Not only do these additions often result in environmental contamination, but also, reserves of the phosphorus rock from where they are obtained are being rapidly depleted. To begin to characterize the genes underlying response of the a major crop to phosphate starvation, Calderon et al. 2008 used microarray analysis to identify a set of genes in maize that were up-or-down-regulated under low phosphorus availability. Although this study gives an important first insight into the molecular basis of the phosphate starvation response in maize, it's difficult to extrapolate the functional importance of a given gene from expression data alone. To further understanding of the maize response to phosphate starvation, I have searched the publically available Uniform Mutator collection to identify insertion of endogenous Mutator transposons into a number of the Calderon et al. candidate genes. I have obtained 16 insertional alleles in a total of 10 candidate genes. Following propagation of segregating stocks, I have optimized PCR primers to detect insertions, and I am in the process of selecting both segregating and homozygous mutant stocks for phenotypic characterization. Although my initial characterization will be greenhouse based, I will initiate also a program of introgression to generate suitable material for testing of mutants under field conditions.

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P320

AC induced rearrangements in the maize P1 gene

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Transposons are DNA segments that can move from one location in the genome to another. As they do so, they usually disrupt gene function. In maize, nearly 85% of the genome is composed of several families of transposable elements, dispersed non-uniformly across the genome (Schnable et al. 2009). This aspect makes maize a very useful model organism for the study of transposons. The maize p1 gene encodes a transcription activator required for the production of the red phlobaphene pigments in the kernel pericarp and the cob, (Grotewold et al. 1991, 1994), and insertion of Ac transposon into this gene affects its phenotypic expression.

To study Ac induced rearrangements in the p1 gene, we screened a large number of ears from a field in which plants of genotype P1-ovov454/p1-ww were pollinated by male plants of genotype p1-ww. The P1-ovov454 allele contains Ac and fAc inserted between exon 3 and exon 2 in the p1 gene and this specifies orange variegated pericarp and cob. Two questions were asked: (i) Has Ac transposition and rearrangement occurred? (ii) If yes, what is the new position of Ac in the genome and what are the flanking sequences? Among a number of putative rearrangements analyzed, one allele (226-3) was of particular interest. Using PCR-based methods to clone the rearrangement breakpoints in 226-3, it was found that Ac and its flanking sequence had undergone an inversion. Other alleles from the same screen are being analyzed and results will be presented in the context of models of alternative transposition involving the Ac and fAc elements. This study enhances our understanding of the Ac transposon system and how transposons can alter the genome. This knowledge may be utilized for plant breeding in future.

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P321

All in one: *TED*, a single gene encodes the transposition functions of a novel autonomous element of the *Mutator* superfamily

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The mutable allele *bz-m175* arose in a transposon trapping experiment of two lines, High Loss and High Knob, known to carry active elements from different families. The transposon in *bz-m175* is an autonomous member of the *Mutator* superfamily, which we have named *TED* (*T*ransposon *E*llen *D*empsey). *TED* is 3960 bp long, ends in 191-bp TIR and causes a 9-bp TSD. *TED* is predicted to encode a 774-amino-acid protein, TEDA, highly homologous to MURA, the *MuDR* transposase. However, unlike *MuDR*, *TED* does not encode a second function (B), which has been postulated to play a role in *MuDR* reinsertion after excision.

To assess if the absence of a B function affected *TED* reinsertion, we have isolated and characterized Bz' germinal revertants from *bz-m175*. Among 15 concordant kernels, 6 represented transposition events. Most Bz' selections were nonconcordant, having arisen from megagametophytic reversions with a high frequency of ~1 per 400 kernels. Reversion to Bz' and *TED* reinsertion in the megagametophytic division that produces the egg and a polar nucleus should lead to a *trTED* element in the *bz-m* embryo of nonconcordant kernels. Indeed, we have found that 2 of 8 nonconcordant *Bz'* have a *trTED* element. Furthermore, among 48 stable *bz-s* derivatives from *bz-m175*, 11 had a deletion at *bz* and a *trTED* element, 7 had a deletion without reinsertion of *TED*, and the remaining 30 had a defective *TED* (*dTED*) element at *bz*. All the *trTED* elements can drive transposition of *dTED*s at *bz*, suggesting that none of the new *trTED* insertion sites are silenced, and that a B function is not required for *TED* excision or reinsertion. Sequence analysis of *trTED* insertion sites showed that *TED* preferentially targets genes, like most other DNA transposable elements, and transposes to unlinked sites, like *MuDR*.

Moreover, we also find that *TED* exhibits duplicative transposition, and forms detectable circularized molecules.

P322

Alternative usage of splice sites augments the transcript diversity of Helitron captured genes between different maize inbred lines

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The propensity to capture and mobilize gene fragments by the highly abundant Helitron family of transposable elements may have significantly impacted the evolution of genes in maize. These elements provide a substrate for natural selection by giving birth to chimeric transcripts intertwining exons of disparate genes. They also capture flanking exons by read through transcription. Here we report expression analysis of selected Helitrons in different maize inbred lines. We recently reported that these Helitrons in inbred B73 produce multiple isoforms of transcripts via alternative splicing (Barbaglia et al., 2012). Despite sharing high degrees of similarity in sequence and insertion site, the splicing profile of Helitrons dramatically differed among various inbred lines. For example, Hel1-331(B73) produces eight isoforms of alternatively spliced transcripts. In contrast, Hel1-331(HP301), Hel1-331(OH7B) and Hel1-331(Tzi8) each encode one transcript isoform in both roots and shoots. The comparison of Helitron sequences identified unique polymorphisms in inbred B73, which potentially gave birth to the alternative spliced sites utilized by transcript isoforms. These observations not only add another level to the creation of transcript diversity by Helitrons among inbred lines but also provide novel insights into the cis-acting elements governing the splice site selection during pre-mRNA processing.

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P323

Comparative analysis of LTR-retrotransposons in *Sorghum bicolor* and its perennial relative *S. propinquum*

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The impact of transposable elements on genome organization and evolution has been of great interest in plant genomics. Studies show that variation in the rate of LTR-retrotransposon accumulation and deletion is responsible for genome size differences among diverse angiosperms. Here, we present a global analysis of LTR-retrotransposon diversity and divergence between *Sorghum bicolor* and *S. propinquum* (PI653737), a wild perennial relative. Although both are closely related, they display marked variation in genome size (*S. bicolor*: ~730 Mb; *S. propinquum* PI653737: 900 Mb). To describe LTR-retrotransposon diversity in *S. bicolor*, LTR_STRUC was used to screen the BTx623 published genome sequence, and the resulting ~7,500 full-length LTR-retrotransposons were annotated and clustered into ~50 known families based on LTR sequence similarity using RepMiner and Cytoscape. *S. bicolor* families contained at the minimum 2 and the maximum 2,751 copies. Approximately 470 “exemplar sequences” that best describe each of the *S. bicolor* families were mined from the total dataset in Cytoscape and queried against the *S. propinquum* Illumina reads using Cufflinks to estimate the relative copy numbers of individual retrotransposons in terms of FPKM. Using this method, there were a total of 33,628 LTRs identified in *S. propinquum* that mapped to 269 of the 470 exemplar sequences, suggesting the absence of LTR-retrotransposons similar to 200 of the *S. bicolor* exemplars. Further, to determine the rates of homologous recombination between LTRs (a well-known mechanisms of DNA removal in plants that results in solo LTRs) the above analysis will be repeated using the reverse transcriptase sequences from each exemplar. These data will be used to determine the copy numbers of full-length vs. solo LTRs, and therefore, calculate rates of DNA removal via this mechanism. Additionally, a similar intra-specific comparative study among several different accessions of *S. bicolor* and *S. propinquum* will be discussed.

Funding acknowledgement: WVU Advanced Energy Initiative

P324

Does the Petunia dTph1 Element Undergo Alternative Transposition?

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Alternative transposition involves the ends of two nearby transposons, and can result in major deletions, duplications, and chromosomal rearrangements. Weil and Wessler (1993) demonstrated that chromosome breakage in maize by Ds elements is a result of Sister Chromatid Transposition. We wanted to determine if alternative transposition is a general phenomenon that can occur with other transposon systems. A good case to test for Sister Chromatid Transposition is found in petunia. Many petunia lines harbor the dTph1 transposon which, like Ac/Ds, is a member of the hAT transposon super family. The S857 allele at the An3 locus in petunia contains two dTph1 elements 30bp apart in opposite orientations (van Houwelingen et al. 1999). The close proximity of the dTph1 elements make this allele a good candidate for alternative transposition. Plants from a line reported to contain the S857 allele were screened for the allele through PCR involving genomic and dTph1 primers. Plants containing the allele were then self-pollinated to obtain homozygous progeny. Progeny found to contain both of the dTph1 elements, in heterozygous and homozygous conditions, were screened for alternative transposition events. The results will be presented.

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P325

Epigenetic regulation of maize transcriptome and TEs activity in response to environmental stresses

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Plant adaptation to environmental cues is (at least in part) achieved by epigenetic mechanisms, as DNA methylation, chromatin modifications and small RNAs, that result in spatiotemporal gene expression changes. Stress-induced epigenetic release may also result in genome destabilization, with the activation and/or the transcription of DNA transposons and retroelements usually silenced. These processes, causing the formation of novel epialleles (stable epigenetic gene variant transmissible to the progeny), can improve the ability of plants to adapt to environmental challenges and can be useful in crop breeding.

To address the role of stress-induced epigenetic gene and TE regulation we are analyzing the salt and drought stresses effects on transcriptional modulation (RNA-seq), transposon activity and on associated smallRNAs (sRNA-seq) and epigenetic marks (ChIP-seq for H3K4me3, H3K9ac and H3K27me3 modifications), both in B73 inbred line and the epiregulator mutant rmr6 (involved in short interfering RNA biogenesis).

Total RNA-seq has been used to compare the transcription of genes, TEs and long non-coding RNAs (lncRNAs) between stressed and control plants, after ten days of stress application and after 7 day of recovery period, both in B73 and rmr6 young leaves. Sequenced reads have been mapped on the maize reference genome to analyze the expression of annotated genes and to create a new annotation of our "stress-specific transcriptome" including TEs and lncRNAs. Gene expression analysis revealed the modulation of many stress-related genes and now we are focusing our attention on transposon and lncRNAs identification and transcription regulation. We are building a genome-wide survey of the stresses effects linking genes and transposons , transcriptional changes with smallRNA population behavior and epigenetic mark distribution. The results obtained by these different approaches aim to identify a robust list of sequences targets of epigenetic regulation (epitargets) that will be further validated in epiregulator mutants, currently under characterization in our lab.

P326

Epigenetic variation through the breeding processes of maize

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Studies of genome-wide genetic changes revealed that highly dynamic genetic changes have been introduced into the maize genome during modern maize breeding over the last four decades. However, the epigenetic changes correlated with remarkable improvement of maize yield remain largely unknown. Here, we performed MethylC-seq and RNA-seq on 4 inbred lines with known pedigree information: 8112, 5003, 478 and Zheng58. Differentially methylated sites (DMSs) in the identity-by-descent (IBD) regions were detected among them. Our current results showed that epimutation rate of DNA methylation are much higher than that of genetic changes in IBD regions. In genic regions, DNA methylation was more variable than in transposable elements. Differentially methylated regions in IBD regions were significantly enriched around the differentially expressed genes among inbred lines examined.

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P327

Epigenetics, the cell cycle and the origin of endosperm

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Several recent studies show that companion cells in flowering plant gametophytes relax epigenetic control of transposable elements (TEs) to promote production of small RNA that presumably assist nearby reproductive cells in management of TEs. In light of this possibility, a closer look at the timing of cell division in relation to angiosperm double fertilization is warranted. From such an analysis, I present a model that helps explain how double fertilization can drive angiosperm evolution by facilitating crosses between genetically diverse parents. A key feature of this model is the order of cell division following double fertilization, since division of the primary endosperm nucleus prior to the zygote would produce small RNA capable of identifying TEs and defining chromatin states in the zygote prior to the zygote entering S-phase of the cell cycle. Crosses between unrelated plants possessing this ability would produce offspring better capable of managing a diverse complement of TEs and their remnants. This model challenges previous notions that the primary purpose of endosperm is for improved nutritional support of the embryo. As well, the model points to a two-step process in angiosperm evolution where endosperm first became the facilitator of distant crosses and later evolved the ability to restrict undesirable crosses through termination of endosperm developmental programming. Together these features bestow on endosperm a direct role in shaping plant genomes.

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P328

Genetic and Epigenetic Control of DNA Methylation Variation in Maize

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DNA methylation is a chromatin mark that can play a role in silencing transposons and in some cases, genes. While DNA methylation is often considered an epigenetic modification, it is a chromatin mark that may be caused by genetic variation or can be independent of genetic variation resulting in true 'epialleles' that may impact heritable variation regardless of genetic state. This distinction is important as genetically-controlled methylation variants will be accounted in GWAS approaches while epialleles are likely to be missed. Genome-wide profiling of DNA methylation in diverse maize lines has identified several thousand differentially methylation regions (DMRs) across the studied genotypes. There are examples of methylation states that are common across multiple genotypes while others are rare, with a single genotype displaying an altered DNA methylation state. In some cases DNA methylation changes are strongly associated with local genetic variation while many other DMRs are not associated with local sequence variation providing evidence for genetic and epigenetic DNA methylation variation. Detailed inspection of some genetically controlled DMRs provides evidence that polymorphic transposable element insertions resulted in DMRs in flanking genomic regions. This study provides evidence for numerous examples of DNA methylation variation caused by both genetic and epigenetic variation.

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P329

Genetic and phenotypic characterization of the *maternal rough endosperm1* (*mre1*) locus

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The maize endosperm accumulates seed reserves of starch and protein and accounts for more than 80% of seed weight. Epigenetic regulation, specifically imprinting within the endosperm, is thought to play an important role in determining endosperm and seed size. However, imprinted genes that regulate maize endosperm size have not been identified. In Arabidopsis, maternal-effect mutants are encoded by imprinted genes. The *maternal rough endosperm1* (*mre1*) locus in maize shows a highly penetrant and expressive maternal-effect on endosperm development. When the female gametophyte is *mre1*, seeds develop a reduced endosperm with a rough surface regardless of pollen genotype. The *mre1* mutant fully transmits through pollen and does not cause seed phenotypes when fertilizing wild-type female gametophytes. Histological analysis of developing *mre1* seeds shows the mutant delays endosperm development as early as 4 days after pollination (DAP) and continues to be delayed through 10 DAP. *mre1* seeds show an aberrant basal endosperm transfer layer and reduction of starch granules in starchy endosperm cells. We mapped *mre1* to a 4 Mbp interval on chromosome 4S with the goal of identifying the *mre1* gene.

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P330

Genetic mapping of *transgene reactivated mutant 1 (tgr1)*, a novel allele of the largest subunit of RNA Polymerase IV in maize.

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Transcriptional gene silencing often arises through the RNA-directed DNA methylation (RdDM) pathway that results in epigenetic changes to the DNA and histones. To further understand the relationship between epigenetic modifications and gene silencing, stably silent transgenic lines containing the b1 genomic transgene (BTG-s) were used in a forward genetic screen to identify transgene reactivated (Tgr) mutants. In the M2 generation, recessive homozygous mutants were identified by purple pigmentation, characteristic of BTG expression. In addition to the loss of transcriptional silencing of BTG-s, *tgr1-1* individuals exhibit hypomethylation of the promoter region of BTG-s and a reduction in 24 nucleotide siRNAs. These molecular phenotypes are consistent with Tgr1 encoding a component of the RdDM gene-silencing pathway in maize. Genetic mapping of Tgr1 indicates that the mutation lies within a 12Mbp interval on Chromosome 1, which also includes the Rmr6 locus. Complementation assays were used to demonstrate that Tgr1 is an allele of Rmr6. We confirmed that *tgr1-1* plants do not contain any of the cloned Rmr6 mutant alleles. Together, these results demonstrate that *tgr1-1* is a novel allele of Rmr6, a gene that has previously been shown to encode the largest subunit of RNA polymerase IV. These genetic results expand the role of Rmr6, and thus Pol IV to include the transcriptional silencing of introduced transgenes.

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P331

Genome wide H3K9me2 methylation profiles in maize highlight associations with DNA methylation

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The dimethylation of lysine nine on histone H3 (H3K9me2) is chromatin modification involved in the regulation of transposable elements and in some cases, gene expression. To investigate the impact of H3K9me2 methylation within maize, we developed a high resolution profile of H3K9me2 levels in B73 through the use of ChIP-seq and for B73 and Mo17 using ChIP-chip profiling. The results from these different methods indicated increased levels of H3K9me2 in the middle portion of maize chromosomes with lower levels in the more gene-rich chromosome arms. H3K9me2 methylation is very rarely observed in genic regions and is much more common in low-copy sequences flanked by transposons. Although B73 and Mo17 display the similar trends of H3K9me2, a number of regions displaying differential H3K9me2 methylation levels were identified, providing evidence for variation in H3K9me2 among genotypes. H3K9me2 levels were also compared to DNA methylation levels in both B73 and Mo17; it was found that high H3K9 methylation levels generally correlated with high DNA methylation levels and that this correlation was conserved through variation across genotypes.

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P332

Imprinting is highly conserved among maize haplotypes

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Imprinting is an epigenetic phenomena that results in the biased expression of alleles based on their parent of origin. Deep sequencing of RNA provides an opportunity to perform genome wide allele-specific expression analysis to detect imprinted genes. Four maize inbred lines were used to generate five pairs of reciprocal hybrid crosses (B73xMo17, B73xKi11, B73xOh43, Mo17xKi11, and Mo17xOh43). RNA was isolated from 14 day after pollination endosperm tissue for each cross and used for RNAseq. Nearly 90% of the genes expressed in endosperm at 14 DAP had enough data and single nucleotide polymorphisms in at least one of the hybrid crosses to assess imprinting. This analysis generated the most complete catalogue of imprinted genes for any flowering plant species to date. Analysis of parent of origin expression patterns identified 108 paternally expressed genes (PEGs) and 69 maternally expressed genes (MEGs). There is evidence for significant, but limited conservation of imprinting between maize, rice and *Arabidopsis thaliana* . The imprinted genes were characterized by analysis of features such as DNA methylation levels, H3K27me3 levels, tissue specific expression patterns, and rates of retention and imprinting of syntenic duplicates. The analysis of imprinting in multiple crosses allows for the assessment of the variation/ conservation of imprinting for different alleles. The majority (87% of MEGs and 95% of PEGs) exhibit imprinting in all crosses examined. Only a small number of genes exhibit allele specific imprinting. The allele specific imprinted (ASI) genes can be used to assess the genetic origin of imprinting.

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P333

Interactions between methylation pathways in intergenic chromatin regulation

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The intergenic genome exhibits multiple forms of chromatin organization that are dependent upon the activity of diverse networks of chromatin modifying enzymes. Two particularly well studied examples are RNA-directed DNA methylation (RdDM) and the chromomethylase/H3K9me2 pathway. Both of these pathways are thought to produce similar outcomes in transcriptional repression of repetitive or foreign DNA, but how they cooperate—or potentially compete—is unclear. In order to understand these specific forms of chromatin regulation better and to expand our view of the maize chromatin landscape, we are profiling chromatin modifications and multiple forms of RNA expression in B73 wildtype and mutant stocks. We recently reported that genes induce RdDM of neighboring transposons, and we have preliminary results suggesting that a version of the chromomethylase/H3K9me2 pathway is also involved with this phenomenon. We are also defining the genomic contexts that favor the chromomethylase/H3K9me2 pathway independently of RdDM, and we are investigating the effects of both pathways on gene expression. In a larger context, we hope through this work to understand the significance of having multiple modes of repressing intergenic transcription and why it is that DNA methylation plays such a central role.

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P334

Investigating the epigenetic specification of maize centromeres

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A suite of poorly understood epigenetic marks specifies eukaryotic centromere function; specific DNA sequences are neither required nor sufficient to recruit kinetochore proteins and form a functional centromere. A dicentric maize (*Zea mays*) chromosome containing a translocated, intact, and inactivated B centromere on the short arm of chromosome 9 (9Bic-1) was previously generated. The supernumery B chromosome centromere provides an excellent tool to study centromere biology because it possesses a B-specific repeat that can be easily tracked. The B centromere of 9Bic-1 originated from the B centromere in the B-A translocation stock TB-9Sb. When canonical B chromosomes are present, the inactive B centromere of 9Bic-1 may undergo non-disjunction during the second pollen mitosis. Occasionally, non-disjunction results in the breakage of 9Bic-1, which may free the inactive B centromere from the active A centromere of chromosome 9. Once released, the inactive B centromere may reactivate and create a heritable minichromosome. Approximately 1400 kernels exhibiting signs of chromosome breakage were screened for reactivated B centromeres using fluorescence *in situ* hybridization. Two independently derived, heritable, minichromosomes containing reactivated B centromeres have been recovered. Therefore, it is possible to compare an active B centromere (TB-9Sb), its inactive descendant (9Bic-1), and its reactivated forms. Because these B centromeres possess the same DNA sequence, epigenetic changes between active, inactive, and reactivated centromeres can be evaluated. RNAseq analyses have been conducted to examine total RNA and small RNA populations from the active, inactive, and reactivated B centromeres.

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P335

Investigating the importance of MITEs as insulators against heterochromatin spreading

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We are investigating the possibility that MITEs (Miniature Inverted-repeat Transposable Elements) protect genes from the spreading of heterochromatin. The maize genome is robust and mostly dominated by heterochromatin due to the abundance of retrotransposons, and heterochromatin can spread from retrotransposons into neighboring areas. Genes are often found bordered by retrotransposons and when spreading occurs, gene expression is inhibited. We have found that when a MITE is present as a boundary between gene and retrotransposon, gene expression is generally higher. Furthermore, the presence of a MITE upstream of a gene is associated with a reduced DNA methylation within the gene in the CG and CHG (H = A, T or C) sequence contexts, suggesting that MITEs can limit the spreading of heterochromatin from nearby retroelements. We are currently analyzing both bisulfite and RNA sequencing data from B73 and mutants lines in the hope of better understanding what features of MITEs may be causing this effect, for instance AT richness or their propensity towards RNA-directed DNA methylation. We are also planning to take advantage of the high presence/absence variation between the B73 and Mo17 inbred lines to compare gene expression with respect to nearby MITEs and retrotransposons. Through this work we hope to expand our understanding of the dynamic interactions between genes and transposable elements in large genome species.

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P336

Maize *Ufo1* modulates tissue-specific small RNA profiles and locus-specific gene expression

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pericarp color 1 (p1) regulates flavonoid biosynthetic genes which produce brick-red phlobaphene in maize. Many *P1* alleles such as *P1-rr* (red pericarp, red cob) and *P1-wr* (white pericarp, red cob) exhibit tissue-specific pigmentation as the result of their differential expression patterns. *Unstable factor for orange 1 (Ufo1)* is a dominant modifier which regulates the expression levels of certain *p1* alleles epigenetically. Compared with the colorless pericarp in *P1-wr* plants, *P1-wr; Ufo1-1* plants exhibit enhanced pigmentation in various tissues, which is associated with hypomethylation of *P1-wr* allele and increased level of *p1* expression. To investigate whether *Ufo1* function is associated with small RNA-directed epigenetic regulation, we conducted extensive small RNA and transcriptome profilings in leaf, tassel, young ear, and pericarp of *P1wr; Ufo1-1* plants. Our results showed a 1.5 fold reduction in the 24 nt abundance only in the *Ufo1-1* pericarp, suggesting that the reduction of 24 nt, silencing small RNAs may contribute to the release of transcriptional repression of genomic loci including *P1-wr* allele in pericarp. The impact of *Ufo1* on small RNA abundance is tissue-specific: while 16% of small RNA-generating loci were repressed by *Ufo1* in pericarp, 5% of loci were repressed in leaf. Furthermore, although majority of the *Ufo1*-impacted small RNA loci were present in more than one tissue type, 20%-40% of *Ufo1*-repressed loci and 30-70% of *Ufo1*-induced loci can be found in one tissue only, and may be associated with certain genome loci. RNA-seq results showed that many genes involved in various biological processes were differentially expressed between wild-type and *Ufo1-1*. In summary, our results indicated that *Ufo1* affects small RNA abundance and gene expression regulation in a locus-specific rather than a global fashion. *Ufo 1* may function in the maintenance of chromatin and DNA methylation state in a tissue-specific manner.

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P337

Methylation Patterns of the Maize *R-stippled* Derivative Lines

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Paramutation occurs between trans-alleles with homologous sequences resulting in a heritable change in gene expression, where epigenetic information from one allele is passed to the other. In maize, the paramutagenic *R* allele, *R-stippled*, silences the paramutable allele, *R-r:standard*, following paramutation. The *R-r:standard* allele is known to show increased methylation following paramutation. The *R* locus is complex, with many alleles composed of multiple highly similar copies of *r1* genes. The *R-stippled* allele, which is composed of four *r1* genes, *Sc*, *Nc1*, *Nc2* and *Nc3*, becomes less paramutagenic as genes are lost. *Sc* alone is not paramutagenic at all. We hypothesized that the *R-stippled* derivative containing two genes would be less methylated than the four-copy allele, particularly in the 5' region of the *Nc* genes. We used sodium bisulfite sequencing to determine the methylation patterns of the different *R-stippled* derivative lines. The analysis of the methylation patterns between the two derivative lines in the 5' region of the *Nc* genes showed no distinct differences in methylation, suggesting that it is not characteristic methylation patterns of the *Nc* genes that is responsible for the differences in paramutagenicity between the two-gene and the four-gene derivatives.

P338

MOP1 impacts the maternal contribution to maize seed

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During double fertilization, the diploid central cell and a haploid sperm give rise to the triploid endosperm. When the ratio of two maternal genome copies and one paternal genome copy is out of balance, seed leathilty is observed. The mature seed phenotypes that arise when individuals with different genomic copy numbers are crossed, or interploidy crosses, can be directly quantifed and exhibit variation between maize lines. Using existing quantitative variation, we attempted a QTL mapping experiment of the terminal seed phenotypes using crosses between tetraploid pollen parents and the IBM (intermated B73 x Mo17 recombinant inbred lines [RILs]). Despite high measured hertabilites, no QTL were detected. In line-cross materials, however, a grandmaternal effect was observed in four replicate experiments. Using an expanded factorial crossing design, no cytoplasmic inheritance was detected. We hypothesize that the epigenetic state of the two parents contributes to the dosage-sensitive mechanisms affecting maize endosperm development. As small RNA (sRNA) have been implicated in genome protection from transposable elements, gene expression, gametophyte development and stress response. To determine if, heritable variation in epigenetic state can impact the parental contribution to maize endosperm we perfomed interpoidy crosses using mutants in MOP1 and MOP2, which are required for proper sRNA biogenesis. No effect was detected with Mop2-1/+ indivduals, however, mop1-1/+ individuals exhibited a decrease in seed survival. MOP1 is necessary for the female contribution to the seed. Triploid offspring from inteprloidy crosses with mop1/+ ears displayed unusual developmental phenotypes that persist throughout the sporophytic phase of plant development that were not obserevd in diploid mop1-1/+ individuals. Together, this indicates sRNA from the female gametophyte contributes to the epigenetic landscape at fertilization. This has consequences for the survival of interploidy crosses in maize. Lastly, the epigenetic state of the embryo can influence the phenotype and architecture of the adult plant.

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P339

Mu killers, old and new

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An important question concerning the function and evolution of genes and genomes is the means by which active transposable elements (TEs) are recognized by their hosts and silenced. *Mu killer* (*Muk*) is a rearranged *MuDR* TE that is competent to heritably silence *MuDR* transposons in trans. It does so as a consequence of the activity of small RNAs derived from a hairpin transcript produced by *Muk*, which causes the formation of heterochromatin at the promoter of *MuDR* elements and subsequent stable epigenetic silencing. We were curious as to whether or not events similar to that which lead to the formation of *Muk* had occurred in the past. To detect such events, we looked for evidence of other *MuDR*-homologous hairpin transcripts. Deep RNAseq revealed the presence of a hairpin RNA with similarity to a MULE (*Mu*-like element) related to *MuDR*. As with *Muk*, this hairpin is associated with 22 nt small RNAs that can potentially target a *mudrA*-like gene carried by the transposon. As with *Muk* smRNAs, production of these 22nt smRNAs does not require the presence of RDR2 (MOP1) and may even be enhanced in the absence of this protein. Several of the MULE elements that are targeted by these 22 nt smRNAs are immediately adjacent to genes. In each of these cases, loss of MOP1 results in an apparent increase in the 22 nt smRNAs and a decrease in expression of the adjacent gene. We present a model for regulation of these and similar genes via small RNA-mediated regulation of associated transposons.

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P340

Nested Insertions and Accumulation of Indels in Coding-MULEs (*Mutator*-like Transposable Elements) are Negatively Correlated with Abundance of MULEs in Maize and Rice

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Mutator-like transposable elements (MULEs) are widespread in plants. MULEs were first discovered in maize where there are a total of 12,900 MULEs in the genome consisting of 2.5 Gb. This is in comparison with rice, which harbors over 30,000 MULEs in a smaller genome (400 Mb). Since maize and rice are close relatives, the differential amplification of MULEs in the two species raised the question that what the underlying mechanism is. We hypothesize this is attributed to different copy numbers of coding MULEs, with the potential to generate transposase that is required for transposition. To this end, we mined the two genomes and detected 530 and 476 MULEs containing transposase sequence (coding-MULEs) in maize and rice, respectively. Over 1/3 of the coding MULEs harbor nested insertions, with similar ratio in the two genomes. Among the maize elements with nested insertions, 32% have insertions in coding regions and over half of them harbor two or more insertions. In contrast, less than 1/5 of the rice elements fall into this category, suggesting that nested insertions in maize are more disruptive. In addition, pair-wise comparison of coding MULEs reveals the presence of significantly more indels among maize elements than rice elements. Taken together, more disruptive nested insertions combined with higher frequency of indels resulted in few (6%) coding MULEs that may encode functional transposase in maize. In contrast, 38% of the coding MULEs in rice retain intact coding regions, which may explain why there are more MULEs in rice than that in maize.

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P341

Paramutation-like interactions between two transgenes in maize leads to cytosine hypermethylation and homology dependent silencing.

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Paramutation is an epigenetic phenomenon that occurs when homologous sequences interact in *trans*, and one allele heritably changes the expression level of the other. In maize, paramutation has been described at several loci involved in the anthocyanin biosynthetic pathway: *r1, b1, pl1,* and *p1* (reviewed by Chandler et al. 2000). The *b1* and *pl1* systems have been used in genetic screens to identify factors required for paramutation, such as the *Mediator of paramutation1*. *mop1-1* mutants are defective in paramutation and transcriptional silencing at distinct endogenous and transgenic loci (Dorweiler et al. 2000; Lisch et al. 2002; McGinnis et al. 2006; Woodhouse et al. 2006).

A two-transgene system was developed to investigate the relationship between chromatin modifications and gene expression. The first transgene encodes a maize DNA methyltransferase translationally fused to a heterologous protein sequence. The second transgene includes a reporter gene adjacent to an operator sequence, designed to interact with the translational fusion protein. Reporter gene expression results in pigmented plant tissue and serves as a visual marker of gene expression. Independent transgenic lines were created for each of these constructs, and crossed to create segregating populations with either one or both transgenes. In plants containing both transgenes, silencing of the reporter gene was observed, corresponding to hypermethylation in the reporter gene promoter and reduced gene expression. However, methylation was also observed at the promoter of the selectable marker, identical in both transgene constructs. Further analysis revealed differential methylation at this promoter in lines with only one transgene. In one construct, this promoter was hypermethylated, and hypomethylated in the other. Subsequent hypermethylation in lines containing both transgenes suggests transgene-to-transgene silencing possibly mediated by small RNAs. Characterization of the mechanisms of this transgene interaction is underway and includes heritability and maintenance of silencing upon outcrossing and in *mop1-1* mutant backgrounds. Progress in these areas will be reported.

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P342

Parent-of-origin effect seed mutants from UniformMu transposon tagging population in maize

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

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P343

Regional mutagenesis of a tandemly duplicated *carbonic anhydrase* gene cluster using the maize transposable elements *Ac*/*Ds*

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The maize transposable elements *Ac* and *Ds* have been used extensively in gene tagging experiments in maize and transgenic systems. Both elements have a tendency to transpose into closely linked sites in the genome, and thus offer unique advantages in gene tagging programs. Here, we describe methods for conducting a regional mutagenesis of a tandemly duplicated gene cluster of *carbonic anhydrase* (CA). CA catalyzes the first dedicated step in C_4 photosynthesis, the hydration of CO_2 into bicarbonate, and is likely rate limiting for carbon fixation in C_4 grasses. We have generated both single and double mutants in several members of the CA gene family using *Ds* insertional mutagenesis. Although three of the CA genes in maize are arranged as tandem duplicates, double mutants were recovered at a high frequency (>1% of plants screened). This was accomplished by remobilizing *Ds* elements that produced single mutants, and then performing a secondary screen to locate new insertions in the adjacent CA gene copies. New insertion events recovered in the second screen contain either a duplicate *Ds* or a footprint at the original donor site, which maintains the knockout of the original gene. Moreover, these double mutants segregate as a single locus due to their proximity. Multiple insertion alleles will allow detailed characterization of each gene family member. Molecular and physiological characterization of the mutants will be discussed. In maize approximately 35% of the genome is represented as tandemly duplicated genes, thus *Ds* tagging offers a versatile approach to the fine-scale genetic dissection of gene function.

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P344

Relationships between H3K27me3 Modifications and Gene Expression

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Plant development is a complicated process that involves interactions between multiple genes. Regulation of expression of numerous plant transcription factors and other genes crucial for plant development is achieved through a variety of means including epigenetic control of gene expression. Epigenetic variation, heritable variation that is not due to DNA nucleotide sequence changes, has been observed in a number of biological phenomena, notably in control of plant and animal development. Despite this widespread interest to epigenetic phenomena, the prevalence and heritable behavior of epigenetic variation is not well understood. Our project attempts to understand the prevalence, heritability, and potential consequences of epigenetic variation in maize. We characterized distribution of one type of epigenetic modifications, trimethylation of histone H3 lysine 27 (H3K27me3) in five distinct tissues in maize and found that this type of epigenetic modifications differs between maize tissues and is likely involved in regulation of maize development and tissue differentiation. H3K27me3 has an inhibitory effect on gene expression likely turning off genes that are not needed for certain tissues. Genes regulated by H3K27me3 are predominantly transcription factors that tend to be expressed in only a small number of tissues. Based on our data, many of the well known maize genes implicated in control of plant development are H3K27me3 targets. Subfamilies of maize transcription factors tend to have similar patterns of H3K27me3, as well as similar patterns of gene expression, further implicating H3K27me3 in controlling gene expression during plant tissue differentiation.

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P345

required to maintain repression5 encodes a DICER-LIKE3 isoform required for both 24nt RNA biogenesis and for paramutation

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Meiotically heritable epigenetic alterations in gene regulation that are influenced by *trans*-homologue interactions (THI) are known as paramutations. Paramutations occurring at both the *purple plant1* (*pl1*) and *booster1* (*b1*) loci are altered by mutations in genes encoding proteins required for 24nt RNA biogenesis. Thus, one working model postulates that 24nt RNAs serve to mediate the THIs triggering paramutation. The *required to maintain repression5* (*rmr5*) locus was identified by four ems-derived recessive mutations that allow strong anthocyanin pigmentation in M2 progeny having a repressed paramutant state of the *Pl1-Rhoades* allele. Positional mapping identified a candidate gene model encoding a DICER-LIKE3 (DCL3) protein having a likely role in 24nt RNA biogenesis. Sequencing identified single transition mutations in this gene model predicting protein dysfunction for each of the *rmr5* mutant alleles. Further, nearly all 24nt RNAs are absent from developing cobs of *rmr5* mutant plants. These data strongly indicate that *rmr5* encodes a DCL3 enzyme, one of potentially two functional DCL3 proteins in maize. The *rmr5* locus is thus synonymous with the *dcl3a* designation. Surprisingly, DCL3a is not required for normal development. Genetic tests indicate that DCL3a is both required to maintain the meiotic repression of paramutant *Pl1-Rhoades* and to establish paramutant states at the *b1* locus. These findings are consistent with the working model that 24nt RNAs derived from RNA polymerase IV and DCL3a are required for the THI affecting inheritance of epigenetic regulatory variation.

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P346

Small RNAs Contribute to Gene Expression Divergence and Inheritance in Maize Hybrids

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Recent studies in maize and other plant systems implicate the epigenetic regulation of transposable elements, often associated with small RNAs, as potential regulators of gene expression variation. Hybridization conditions genome-scale changes in gene expression, and provides an excellent system to study the inheritance of gene expression. We conducted an integrated mRNA and small RNA profiling study of developing earshoots of the B73 and Mo17 inbred lines, their reciprocal hybrids, and near-isogenic version of these four genotypes carrying the *mop1* mutation, which conditions a global reduction of 24-nt small RNAs. We found that ~19% (3,759) of the detected genes showed expression divergence among the four genotypes, nearly all of which could be attributed to expression variation between the parents. About half of these differentially expressed genes were expressed at mid-parent levels in the hybrids, with the remainder expressed at levels consistent with dominance (either high-parent or low-parent). We observed that genes exhibiting the high-parent inheritance pattern were enriched in functions related to growth, whereas genes associated with stress response and cell death were overrepresented among those showing low-parent inheritance. The *mop1* mutation did not significantly alter the expression variation observed between the B73 and Mo17 parents, but had a major impact on the inheritance patterns of more than 50% of the differentially expressed genes. We also discovered that genes within genomic regions previously shown to be targets of domestication or improvement, which exhibit reduced degrees of expression variation, are enriched for small RNAs and are more likely to show non-additive inheritance in hybrids. Collectively, our results suggest that genes with expression divergence among parents which also exhibit dominant inheritance may contribute to the greater growth and stress tolerance of hybrids, and that the amplification of 24-nt small RNAs by the MOP1 RNA-dependent RNA polymerase helps maintain established inheritance patterns of gene expression.

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P347

The glossy13 gene encodes a putative ABC transporter

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Insertional mutagenesis is used to facilitate gene cloning in a variety of organisms. In maize the *Mu* transposon is the most widely used insertional mutagen. Typically, *Mu*-active maize lines carry more than a hundred copies of *Mu*. To overcome this complexity, we developed SeqWalking, a novel genome walking strategy. We utilized SeqWalking to clone the *glossy13* gene, which is involved in the accumulation of cuticular waxes. Cuticular waxes play important roles in regulating water loss and interactions with pathogens. Using genetic mapping data and expression data from a BSR-Seq (Liu et al., PLOS ONE, 2012) experiment, the GRMZM2G118243 gene was identified as a strong candidate for *glossy13*. Multiple EMS-induced alleles of *glossy13* contain premature stop codons in GRMZM2G118243, demonstrating that this gene is indeed *glossy13*. GRMZM2G118243 is an ortholog of AtABCG32 (*Arabidopsis thaliana*), HvABCG31 (barley) and OsABCG31 (rice) Consistent with our cloning data, AtABCG32 encodes an ABCG subfamily transporter involved in the export of cuticular components from epidermal cells (Makarevitch al., PLOS ONE, 2012); HvABCG31 and OsABCG31 contribute to the formation of cutin (Chen, et al., PNAS, 2011).

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P348

The initial characterization of *chr120* point mutation alleles.

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Epigenetic modifications, including DNA methylation, are associated with changes in gene expression. Hypermethylation of promoter regions is associated with gene silencing while their hypomethylation is associated with gene expression. The Arabidopsis (*Arabidopsis thaliana*) *morpheus' molecule1* (*mom1*) mutant exhibits a loss of transcriptional silencing without the loss of associated DNA methylation in the promoter region. Maize (*Zea mays*) *Chr120* has been determined to be an ortholog of *MOM1*, but function of this protein in maize is not known. We acquired four maize lines with mutant *chr120* alleles from TILLING (Targeting Induced Local Lesions IN Genomes) populations generated by EMS mutagenesis. The first TILLING allele (*chr120-L646F*) we studied exhibited an albino phenotype. We observed some evidence for lethality for two of the other alleles, while the third did not demonstrate evidence for lethality or albinism. As EMS mutagenesis generates multiple mutations, we also considered *chr120-L646F* plants having a second mutation in *W11*. The mutant allele of the tightly linked *W11* has been previously shown to give an albino phenotype. Our complementation analysis has determined that *W11* and *Chr120* however are neither allelic nor there is a second mutation in *W11* in the *chr120-L646F* background. Because of its orthology with *MOM1*, we hypothesized that *Chr120* might be an epigenetic regulator of gene expression. We tested this hypothesis by studying the affects of homozygous *chr120-L646F* on the epigenetically, transcriptionally silenced *B1* transgene (BTG). Our preliminary results indicate that *chr120-L646F* does not relieve silencing of BTG. Future research will focus on identifying the gene responsible for the albino phenotype, and determining its interaction with *Chr120*.

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P349

The maternal epigenome influences small RNA profiles of its progeny

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The potential for epigenetic diversity between distinct parental lines is large, particularly in genomes with high transposable element (TE) content (>85% of the maize genome) targeted by a small RNA-directed DNA methylation (RdDM) pathway. RdDM utilizes 24-nucleotide small RNAs (24-nt sRNAs) as vectors of epigenetic information that guide *de novo* cytosine methylation. We hypothesize that interactions between parental sRNA populations in hybrids serve as a level of regulatory diversity affecting heterosis. Certain behaviors of the *Rhoades* allele of the *purple plant1* locus (*Pl1-Rhoades*) that is a target of RdDM control serve as a single locus example of heterosis: combining epigenetically repressed *Pl1-Rhoades* with structurally distinct *pl1* alleles produce hybrids with derepressed *Pl1-Rhoades*. Motivated by this model, we are evaluating the importance of RdDM and/or 24nt-sRNAs in defining the epigenetic status of larger hybrid regions around *Pl1-Rhoades*. We introgressed both *Pl1-Rhoades* alone and *Pl1-Rhoades* in linkage with a mutant allele (*required to maintain repression1-1*; *rmr1-1*) disrupting RdDM into B73. These stocks (*Pl1-Rhoades^B73* and *Pl1-Rhoades rmr1-1^B73*) served as female parents in crosses by B73, thus limiting the genetic differences between parental chromosomes in the F1 to regions around *Pl1-Rhoades* and *rmr1-1*. We then compared deep-sequenced sRNA profiles of immature ears between parents and hybrids. In the absence of RMR1 function, 24-nt sRNA levels are decreased. The resulting hybrid (*Pl1-Rhoades rmr1-1^B73 / B73*) also has significantly decreased 24-nt sRNA levels compared to the other hybrid (*Pl1-Rhoades^B73 / B73*). Importantly, these decreased sRNA levels in the *Pl1-Rhoades rmr1-1^B73 / B73* hybrid are perpetuated throughout plant development in the presence of a fully functional RdDM pathway. This observation supports the concept of small RNAs as maternally provided cytoplasmic vectors of heritable epigenetic information and indicates that epigenetic diversity in the parents can influence hybrid genome function. We are now using the sRNA profiles to probe how differences in the parental epigenomes affect specific loci in the progeny.

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P350

The Presence of Activator (Ac) Elements in Nonspotted Kernels Produced by Transposition of Seven Maize Ac Elements Located on the Short Arm of Chromosome 1.

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Last year we reported the results of performing genetic tests of the fate of seven mapped Ac elements following their transposition. Transposition events were scored as the low frequency near-colorless kernels among the mostly coarsely spotted kernels on ears borne on plants homozygous for the Ac element that were crossed by a homozygous r1-scm3 tester. The Ac elements and the r1-scm3 were all in near isogenic color converted W22 inbred lines. The near-colorless kernels were either near-colorless fine spotted kernels or nonspotted kernels. Whereas the spots on the fine-spotted kernels demonstrated the presence of an Ac element at an increased dosage, the nonspotted kernels could manifest either the presence of Ac elements at a high dosage, or the absence or silence of an Ac element. In order to distinguish the nonspotted kernels with a high dosage of Ac from those lacking an active Ac element we genetically tested 209 plants among 232 plants grown from nonspotted kernels; Ac was present in 85 (40.7%) of the plants but not present in 124 (59.3%) of the plants. The genetic tests used crosses with the r1-scm3 tester in order to dilute the Ac copy number. Using PCR primers specific for each of the Ac elements at its original site on chromosome 1, we found that Ac was present at its original site in 57 (24.6%) and absent in 175 (75.4%) of the plants. The combined results of the two tests show Ac was present in 121 (52.2%) of the plants and possibly absent in 111 (47.8%) of the plants. A negative test result does not preclude the presence of an Ac element at a site in the genome where it does not transcribe a transposase message nor does it preclude a negative genetic test result because of a very high copy dose of Ac.

P351

Transgenerational inheritance of epigenetic regulation by *Unstable factor for orange1 (Ufo1)* in maize

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Epigenetics is the study of heritable changes in gene expression or cellular phenotype that are attributable to mechanisms other than changes in DNA sequence. This study is designed to investigate the transgenerational inheritance of *Ufo1*-induced changes in maize. The maize *pericarp color1 (p1)* gene is being used as a reporter for the *Ufo1*-induced phlobaphenes phenotypes. In the presence of *Ufo1-1* mutation, *p1* is hyperactivated and thus phlobaphenes are ectopically accumulated throughout the plant body. Previous studies have shown that *Ufo1-1*-induced pigmentation phenotypes are only observed in a subset of *P1-wr; Ufo1-1* plants. Interestingly, within this subset, pigmentation level is highly variable. Also, it has been shown that this increased pigmentation is associated with changes of DNA methylation pattern in the *P1-wr* distal enhancer and intron sequences. Moreover, the increased pigmentation phenotypes in backcross population are accompanied by progressive loss of *P1-wr* methylation from one generation to the next. Thus, the objective of this study was to investigate the inheritance of *Ufo1-1* through the genotyping results using linked markers. This information was then compared to the *Ufo1-1*-induced phenotypes to verify the epigenetic regulation of *P1-wr* by *Ufo1-1*. The second objective of this study was to establish qRT-PCR based assay to investigate DNA methylation level at *p1* in different genotypes of *P1-wr; Ufo1-1* plants. Methylation dependent restriction enzyme, *MspJ*I, was used to treat the genomic DNA followed by qRT-PCR quantification using primers flanking the methylation sensitive sites on the distal enhancer of *p1*. The relative methylation levels provide better understandings on the correlation between the range of pericarp pigmentations in *P1-wr; Ufo1-1* plants and their respective DNA methylation states at *p1*. The understanding of *Ufo1-1* mediated regulation can be a foundation for understanding epigenetic transgenerational inheritance.

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P352

UniformMu insertions in gene for Exocyst 70 subunit correlate with empty pericarp phenotype

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Carbon partitioning includes the processes by which the products of photosynthesis are distributed throughout the plant. To gain a better understanding of which genes are involved in these processes, we studied maize lines from the UniformMu population segregating for defective kernel phenotypes. Using Mutator (Mu) transposon insertion mapping data from previous generations, Mu insertions in candidate genes were assigned to each family. PCR was used to test which Mu insertions co-segregated with the defective kernel phenotypes. An insertion in a gene encoding an Exocyst 70 (Exo 70) subunit protein segregated with an empty pericarp phenotype, indicating a putative role for this gene in kernel development. There are hundreds of Exocyst complexes in the plant cell, and each subunit may have a distinct function. Some Exo70 proteins act as tethering proteins, which are involved in vesicle docking during exocytosis. Others are implicated in pre-mRNA splicing, cell differentiation, and pollen tube growth. Further research will be directed towards determining the precise role of the Exocyst 70 protein in kernel development. Additional UniformMu alleles are currently being examined to confirm that the mutation in Exo70 is responsible for the phenotype.

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P353

Global run-on sequencing identifies transcriptional control of the maize genome by RNA polymerase IV

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RNA polymerase IV (Pol IV) has been exapted in domesticated maize to canalize the proper developmental expression patterns of specific haplotypes controlling particular morphological traits [1,2]. The nature of this regulatory control remains unclear. Although derived from Pol II, Pol IV's ability to transcribe RNA is highly compromised [2]. Pol IV interferes with the ability of Pol II to transcribe certain LTR retrotransposons (RT) [3]; this observation leads to a working model in which specific haplotypes are transcriptionally regulated by Pol IV via competitions with Pol II [3,4]. Pol IV also controls the biogenesis of 24nt RNAs having the capacity to define cytosine methylation (5meC) patterns [5] but these patterns are unlikely responsible for developmental canalization as other mutants deficient for 24nt RNAs are phenotypically normal [5,6,7, see Meeting Poster regarding DCL3a from Narain et al.].

We used global run-on sequencing (GRO-seq) to define the nascent RNA transcriptome of maize inbred B73 in both the presence and absence of Pol IV as a first step towards identifying relevant haplotypes for further study. In general, only a fraction of the non-coding intergenic space is transcribed with a strong bias for unclassified LTR RTs indicating that most of the maize genome remains transcriptionally inert even in the absence of Pol IV. Significant antisense transcription of genic regions was also found. Statistical analyses indicate that 204 genic regions are differentially transcribed between non-mutant and Pol IV mutant siblings. Surprisingly, ~1/3 of all genic transcription repressed by Pol IV action is in antisense orientation indicating that Pol IV often serves to prohibit read through transcription from adjacent 3' promoters. Additional cases of increased LTR RT transcription were found in the absence of Pol IV, consistent with the RNA polymerase competition model. These studies represent the first application of GRO-seq in plants, providing a novel means to understand transcriptional control.

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P354

Inverted duplication alleles generated by Ac-induced Sister Chromatid Transposition (SCT) at p1 locus show repressed Ac activity and reduced levels of Ac transcript

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Previous research demonstrated the model of Sister Chromatid Transposition (SCT) by directly-oriented Ac ends at the maize p1 locus (Zhang 1999). The transposition involves the 5' and 3' termini of Ac elements on sister chromatids. Insertion of the excised non-linear transposon back into chromosome 1S generates inverted duplications flanking the p1 locus, and additional insertions with complex structures at various target sites. The complex insertions can contain from zero to two additional copies of full Ac elements or partial Ac fragments which may impact Ac expression. Here we identified an additional three inverted duplication alleles (p1-ww-id) derived from SCT. These p1-ww-id alleles contain duplications ranging in size from 0.4 to 3 Mb, and complex insertions with various Ac configurations. We tested the Ac activity by crossing each allele with Ac tester lines (p-wr, r-m3:: Ds); the resulting kernels have small purple aleurone sectors indicating late Ds excision from the r1 gene. Analysis of Ac RNA by qRT-PCR shows that the p1-ww-id alleles have a significantly lower level of Ac transcript compared to the progenitor allele. These results suggest that Ac transcription is repressed or the Ac transcript is destabilized, possibly by the unique Ac configurations present in the duplication/complex insertion alleles. The structures and the Ac activity of the inverted duplication alleles will be presented, and possible mechanisms of Ac repression will be discussed.

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Late Submissions

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Influence of chloride load on the L-proline amount of maize callus tissues

(submitted by KV Derkach <katerina-d-d@yandex.ua>)
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Salinity of soil and soil waters are actual problems of land utilization. Chloride salinity is the most common kind, in Ukraine it is dominated by sulphate and carbonate forms. Chloride salinity has a super-negative effect on the maize plant. Protective plant response to the negative effect of abiotic factors is being induced by a lot of cell systems. One of the responses to the stress factors (salinity, drought and low temperatures) is the accumulation of free L-proline in the cells. Proline is an important cell osmoprotector. The subject of our work includes the determination of proline amount in maize callus tissues under chloride load, the characterization of influence of sodium chloride on the regeneration potential of callus tissues. Research material was represented by 5 inbreds of maize commercially valuable Lancaster germplasm (DK633/266, DK633/325, DK236, DK3070, DK6080) and inbred of Polish germplasm (PLS61). Primary explants for induction of callus tissues were immature embryos, 1.5 mm in length. Chloride load in vitro was simulated by adding into the medium for subcultivation sodium chloride in concentrations of 6, 30 and 60 g/l. The content of L-proline was determined for 330-day stabilized maize callus tissues. Determination of the proline amount was performed by a modified method [Bates, 1963]. The experimental data allow concluding that the response of maize callus tissues to chloride load leads to the accumulation of proline. The content of proline in callus tissues depended on the concentration of sodium chloride in the nutrient medium and increases with its magnification. Concentrations of sodium chloride in the nutrient medium of 30 and 60 g/l completely suppress regenerative potential of maize callus tissues, while 6 g/l sodium chloride permits the plant regeneration of certain genotypes.

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