53rd Annual Maize Genetics Conference

Program and Abstracts

March 17 – March 20, 2011

Pheasant Run
St. Charles, Illinois

This conference received financial support from:

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BGI | KBioScience | LemnaTech | Sequenom

We thank these contributors for their generosity!

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Cover art by

Thomas Kono
Department of Plant Sciences, UC Davis

General Information

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: During poster sessions and 9:00 PM to Midnight: 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The maize meeting is a forum for presentation and discussion of unpublished material.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/2011/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 2011 Steering Committee:

Erik Vollbrecht, Chair (vollbrec@iastate.edu)
Uta Paszkowski, Co-chair (uta.paszkowski@unil.ch)
Paula McSteen, Treasurer (mcsteenp@missouri.edu)
Robert Bensen (robert.bensen@syngenta.com)
Mark Cigan (mark.cigan@pioneer.com)
Jane Dorweiler (jane.dorweiler@marquette.edu)
John Fowler (fowlerj@science.oregonstate.edu)
Karen Koch (kekoch@ufl.edu)
Nathan Springer (springer@umn.edu)
William Tracy (wftracy@wisc.edu)
Marty Sachs, local organizer, ex officio (msachs@uiuc.edu)
Mary Schaeffer, abstract coordinator, ex officio (Mary.Schaeffer@ars.usda.gov)
Carson Andorf, abstract coordinator, ex officio (Carson.Andorf@ars.usda.gov)

Acknowledgements

Many thanks go to Carson Andorf and Mary Schaeffer for their tremendous efforts in organizing and assembling 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. Thanks go to Kathy Newton for her past stewardship in managing the finances for this meeting and Mark Cigan, Robert Bensen, Paula McSteen, Uta Paszkowski, and Erik Vollbrecht for their efforts in securing funding to support graduate student attendance at this meeting. Finally, many thanks go to Marty Sachs for his wisdom in all things related to the Maize Meeting.

Schedule of Events

Thursday, March 17

3:00 PM – 6:00 PM	**REGISTRATION / POSTER HANGING**
6:00 PM – 7:00 PM	**DINNER**
7:00 PM – 7:15 PM	**WELCOME AND ANNOUNCEMENTS**
7:15 PM – 9:00 PM	**SESSION 1 – PLENARY TALKS** Chair: Erik Vollbrecht
7:15 PM	**Elizabeth Lee, University of Guelph** *Grain Yield, the known knowns and the known unknowns ...*
8:05 PM	**Simon Chan, UC Davis** *Engineering centromeres to produce haploid plants*
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING / REGISTRATION**

Friday, March 18

7:00 AM – 8:00 AM	**BREAKFAST**
8:00 AM – 8:15 AM	**ANNOUNCEMENTS**
8:15 AM – 10:15 AM	**SESSION 2 - TRANSPOSONS, EPIGENETICS AND DOMESTICATION** Chair: Karen Koch
8:15 AM	**Anthony Studer, University of Wisconsin-Madison** *A Transposon Insertion was the Causative Mutation in the Maize Domestication Gene tb1*
8:35 AM	**Jose Gutierrez-Marcos, University of Warwick** *Large-scale epigenetic reprogramming during maize seed development*
8:55 AM	**Hong Li, University of California, Berkeley** *Coordinate regulation of multiple targets of epigenetic silencing during maize development*
9:15 AM	**Gregory Downs, University of Guelph** *Identification and Characterization of Gene Coexpression Networks in Maize Development*
9:35 AM	**James Schnable, University of California, Berkeley** *The two different genomes within maize: differences in post-WGD gene loss, PAV, expression, and classical maize genes*
9:55 AM	**Matthew Hufford, University of California, Davis** *Genome-wide effects of domestication and improvement in landraces and modern maize*
10:15 AM	**BREAK**
10:45 AM – 12:25 PM	**SESSION 3 – CYTOGENETICS AND KERNEL DEVELOPMENT** Chair: Mark Cigan
10:45 AM	**Hank Bass, Florida State University** *SUN (Sad1p/Unc-84) Domain Genes Of Maize: Evidence for a Small Gene Family of Two Ancient Isoforms*
11:05 AM	**Ashley Lough, University of Missouri** *Mitochondrial DNA Sequences in the Nuclear Genomes of Diverse Maize Lines*
11:25 AM	**Elizabeth Takacs, Cornell University** *Analyses of shoot meristem organization during maize embryo development*
11:45 AM	**Masaharu Suzuki, University of Florida** *VP8 and BE1, two distinct membrane-localized proteins, regulate embryo development and lateral organ numbers.*
12:05 PM	**Philip Becraft, Iowa State University** *A negative regulator and duplicate IDD transcription factors control aleurone cell differentiation*

Friday, March 18 (continued)

12:30 PM – 1:30 PM **LUNCH**

1:30 PM – 5:00 PM **POSTER SESSION 1**

Presenters should be at odd numbered posters from 1:30 PM to 3:00 PM.
Presenters should be at even numbered posters from 3:00 PM to 4:30 PM.
Beverages will be available from 3:30 PM to 5:00 PM.

5:00 PM – 6:15 PM **SESSION 4 – RESOURCES FOR MAIZE GENETICS, GENOMICS AND BIOLOGY**

Chair: Nathan Springer

5:00 PM **Donald McCarty, University of Florida**
New mutants in accessible UniformMu seed stocks

5:12 PM **Alice Barkan, University of Oregon**
Use of Illumina Sequencing to Identify Transposon Insertions Underlying Mutant Phenotypes in High-Copy Mutator Lines of Maize

5:24 PM **Justin Walley, University of California, San Diego**
Peptide-Driven Gene Models and Protein Atlas of the Developing Maize Seed

5:36 PM **Hilde Nelissen, Flanders Institute of Biotechnology and Gent University**
The maize leaf: an ideal tool to study cell division and cell expansion, the processes driving growth

5:48 PM **Carson Andorf, United States Department of Agriculture - Agriculture Research Service (USDA-ARS)**
Reinventing MaizeGDB

6:00 PM **Jer-Ming Chia, Cold Spring Harbor Laboratory**
Maize HapMapV2 - Capturing variation in a genome in flux

6:30 PM – 7:45 PM **DINNER**

8:00 PM – 1:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY**

Saturday, March 19

7:00 AM – 8:15 AM	**BREAKFAST**
8:15 AM – 10:15 AM	**SESSION 5 – QUANTITATIVE GENETICS AND SYSTEMS BIOLOGY** Chair: Jane Dorweiler
8:15 AM	**Jagdeep Kaur, Donald Danforth Plant Science Center** *Toward Fusarium ear rot resistant fumonisins-free transgenic maize for sub-Saharan Africa*
8:35 AM	**Judith Kolkman, Cornell University** *Comprehensive association, linkage and mutant analysis implicate Tasselseed2 in defense response in maize*
8:55 AM	**Guri Johal, Purdue University** *Integrating MAGIC and NAM to enrich the genetic network underlying the maize immune response*
9:15 AM	**Pat Schnable, Iowa State University** *Genome-wide patterns of genetic variation among elite maize inbreds*
9:35 AM	**Richard Johnson, University of Illinois** *Ear Shoot Meristem Transcript Abundance and Grain Yield*
9:55 AM	**Andrea Eveland, Cold Spring Harbor Laboratory** *A systems approach to elucidating transcriptional networks that control maize inflorescence architecture*
10:15 AM – 10:45 AM	**BREAK W/ BEVERAGES**
10:45 AM – 12:25 PM	**SESSION 6 – DEVELOPMENTAL AND CELL BIOLOGY** Chair: Paula McSteen
10:45 AM	**Charles Hunter, University of Florida** *A narrow-leaf warty phenotype and cell division anomalies result from mutations in maize Cellulose Synthase-like D1*
11:05 AM	**Jihyun Moon, University of California, Berkeley** *The grass specific liguleless narrow gene and its paralog regulate leaf development in maize*
11:25 AM	**Jong-Jin Han, Cold Spring Harbor Laboratory and Stony Brook University** *The maize mutant Tunicate1 is caused by ectopic expression of a MADS-box transcription factor, Zmm19 gene in a dosage-dependent manner*
11:45 AM	**Xiang Yang, Iowa State University** *RAMOSA1 interacts with KNOTTED1 and regulates meristem determinacy via gibberellins during maize inflorescence development*
12:05 PM	**Peter Bommert, Cold Spring Harbor Laboratory** *Natural variation and new pathways in maize meristem size regulation*
12:30 PM – 1:30 PM	**LUNCH**

Saturday, March 19 (continued)

1:30 PM – 5:00 PM **POSTER SESSION 2**

Presenters should be at even numbered posters from 1:30 PM to 3:00 PM.
Presenters should be at odd numbered posters from 3:00 PM to 4:30 PM.
Beverages will be available from 3:30 PM to 5:00 PM.

5:00 PM – 6:00 PM **COMMUNITY SESSION - Maize Genetics Executive Committee**

6:00 PM – 7:00 PM **DINNER**

7:15 PM – 9:00 PM **SESSION 7 – PLENARY TALKS**

Chair: Uta Paszkowski

7:15 PM **Ron Phillips, University of Minnesota**
Good science in maize genetics: is serendipity involved?

8:05 PM **Ueli Grossniklaus, University of Zurich**
Epigenetic control of seed development in Arabidopsis

9:00 PM – 2:00 AM **INFORMAL POSTER VIEWING & DANCE**

Sunday, March 20

7:00 AM – 8:20 AM	**BREAKFAST**
8:20 AM – 8:30 AM	**ANNOUNCEMENTS**
8:30 AM – 9:50 AM	**SESSION 8 – BIOCHEMICAL GENETICS / FUNCTIONAL GENOMICS I** Chair: Bob Bensen
8:30 AM	**Xinhui Shi, University of Toledo** *The roles of ZmMYB31 and ZmMYB42 in the regulation of the maize lignin biosynthetic pathway*
8:50 AM	**Rajeev Gupta, Pioneer Hi-Bred International, Inc.** *Engineering N Storage Capacity of Plants to Improve NUE*
9:10 AM	**Yuanxin Yan, Texas A&M University** *Diverse roles of jasmonic acid in reproductive development and defense as revealed by the analyses of the JA-deficient OPR mutants*
9:30 AM	**Federico Martin, University of Florida** *Maize threonine synthase mutants cause an embryo-lethal, rough endosperm phenotype*
9:50 AM	**BREAK**
10:20 AM – 11:30 AM	**SESSION 8 – BIOCHEMICAL GENETICS / FUNCTIONAL GENOMICS II** Chair: John Fowler
10:20 AM	**Nengyi Zhang, Cornell University** *Genome-wide association study of carbon and nitrogen metabolism in the maize nested association mapping population*
10:40 AM	**Annett Richter, Martin Luther University Halle** *Identification of regulatory elements of terpene biosyntheses by Nested Association Mapping (NAM) and Genome Wide Association Study (GWAS)*
11:00 AM	**Sanzhen Liu, Iowa State University** *Segregation of Non-allelic Homologs Generates Transgressive Variation in Genome Content*
11:30 AM	**ADJOURNMENT**

Posters

Bioinformatics & Computational Biology

P1	**Marcela Monaco** <mmonaco@cshl.edu>	*Gramene: A Resource For Comparative Plant Genomics*
P2	**Cesar Alvarez-Mejia** <calvarez@ira.cinvestav.mx>	*In search of new genomic regions involved in maize domestication*
P3	**Kui Xiang** <ninuokui@163.com>	*A meta-analysis of QTL associated with ear rot resistance in maize*
P4	**Jon Beck** <jbeck@truman.edu>	*Automated Functional Annotation of Maize Genes Using a Support Vector Machine*
P5	**Hainan Zhao** <zhaohainan@cau.edu.cn>	*De novo assembling of Mo17 genome using short reads from Solexa sequencing*
P6	**Karen McGinnis** <mcginnis@bio.fsu.edu>	*Development of a computational pipeline to identify and classify non coding RNAs in maize*
P7	**Zhou Fang** <fang0157@umn.edu>	*Evidence for megabase-scale inversion polymorphisms in wild and domesticated Zea mays based on SNP genotyping data*
P8	**Tanja Pyhäjärvi** <tpyha@ucdavis.edu>	*Genomic effects of local adapation in Zea mays ssp. parviglumis populations*
P9	**Jeff Glaubitz** <jcg233@cornell.edu>	*Improving the B73 reference genome via genotyping by sequencing (GBS)*
P10	**Justin Fincher** <fincher@cs.fsu.edu>	*Using a Support Vector Machine to Predict Nucleosome Occupancy Likelihood (NOL) in the Maize Genome*
P11	**Xin Meng** <xin.meng@pioneer.com>	*Maize global transcriptomics reveals pervasive leaf diurnal rhythms but rhythms in developing ears are largely limited to the core oscillator*
P12	**Shiran Pasternak** <shiran@cshl.edu>	*The Maize Genome Project: Past, Present, and Future*
P13	**Kranthi Varala** <kvarala2@uiuc.edu>	*Rapid genotyping of soybean cultivars using high throughput sequencing*
P14	**John Fowler** <fowlerj@science.oregonstate.edu>	*RNA-seq Reveals Novel Aspects of the Maize Gametophytic Transcriptomes*
P15	**Eric Lyons** <ericlyons@email.arizona.edu>	*Pan-grass synteny: reciprocal gene deletion did not drive the radiation of the major grass species*
P16	**John Gray** <jgray5@utnet.utoledo.edu>	*Phylogenomic analysis of the Trihelix transcription factor family in grasses.*
P17	**Brian Smith-White** <smtwhite@ncbi.nlm.nih.gov>	*Plant Genomic Resources at National Center for Biotechnology Information*
P18	**Taner Sen** <taner.sen@ars.usda.gov>	*Sequence, Structure and Dynamics Analysis of Thermostability in Endoglucanases*
P19	**Carson Andorf** <carson.andorf@ars.usda.gov>	*Reinventing MaizeGDB*
P20	**Bremen Braun** <bremen.braun@ars.usda.gov>	*The MaizeGDB Genome Browser: Tools and Resources*
P21	**Ethalinda Cannon** <ekcannon@iastate.edu>	*POPcorn: A PrOject Portal for corn*
P22	**Lisa Harper** <ligule@berkeley.edu>	*The new MaizeGDB video tutorial page*

P23 **Mary Schaeffer** <mary.schaeffer@ars.usda.gov> — *MaizeGDB Curation Activities -- Diverse Genomes, Gene Expression, Community GO Annotation*

P24 **Darwin A Campbell** <darwin.campbell@ars.usda.gov> — *MaizeGDB virtualized infrastructure*

P25 **Jon Duvick** <jduvick@iastate.edu> — *Insertional Mutagenesis In Maize Using Ds: Web Resources and Tools at PlantGDB*

P26 **Jon Duvick** <jduvick@iastate.edu> — *Who Moved My Exon? Improving Gene Structure Annotations for Maize*

Biochemical Genetics

P27 **Pamela Peña** <pamela.pena@huskers.unl.edu> — *Modulating Nitrogen Flux in Sorghum and Wheat*

P28 **Kevin Chu** <chu16@purdue.edu> — *A Mechanistic Basis for Adult Plant Resistance in the Maize-CCR1 Pathosystem*

P29 **Marilyn Warburton** <marilyn.warburton@ars.usda.gov> — *A public platform for the verification of the phenotypic effect of candidate genes for resistance to aflatoxin accumulation and A. flavus resistance in maize*

P30 **David Pan** <dpan@wisc.edu> — *An endosperm enzyme catalyzes the formation of phosphotrieser and phosphdiester bonding complex between nucleic acids with altering their stucture*

P31 **Yongrui Wu** <yongrui@waksman.rutgers.edu> — *Basis and Selection for Quality Protein Maize (QPM)*

P32 **Xiaomei Guo** <xguo3@unl.edu> — *Characterization of the Expression and Function of Pyrophosphate Dependent Phosphofructokinase (PFP) in Endosperm Maturation in Quality Protein Maize*

P33 **Alan Myers** <ammyers@iastate.edu> — *Control of Granular Starch Accumulation by Starch Synthase III when Isoamylase-type Starch Debranching Enzyme is Compromised*

P34 **Michael Lewis** <mwlewis@berkeley.edu> — *Control of cell fate acquisition during maize leaf development*

P35 **Jia Wei** <jwrf4@mail.missouri.edu> — *Developing a Bacterial Model for a Mitochondrial Defect*

P36 **Peter Balint-Kurti** <peter_balintkurti@ncsu.edu> — *Developing a robust Virus-induced gene silencing (VIGS) system for maize.*

P37 **Gertraud Spielbauer** <gspielbauer@ufl.edu> — *Dosage-dependent genes affecting seed composition or weight*

P38 **Brent O'Brien** <bob2373@ufl.edu> — *Expression Analysis of the Cellulose Synthase (CesA) Gene Family Indicates that CesA10, CesA11, and CesA12 are Strongly, Though Not Exclusively Associated with Secondary Cell Wall Biosynthesis.*

P39 **Iffa Gaffoor** <sig2@psu.edu> — *Flavonoid mediated resistance to anthracnose leaf blight in sorghum and transfer of this trait into maize*

P40 **Patrick Breen** <pbreen@purdue.edu> — *Genetic Control of Starch Digestion: Better Food & Fuel*

P41 **Robert Bruce** <rbruce@uoguelph.ca> — *Genetic and physiological characterization of a wilted Zea mays mutant*

P42 **Sandeep Marla** <smarla@purdue.edu> — *Genetic basis of adult plant resistance in maize to a lethal leaf blight and ear mold pathogen*

P43 **Nengyi Zhang** <nz45@cornell.edu> — *Genome-wide association study of carbon and nitrogen metabolism in the maize nested association mapping population*

P44 **Claudia Lenk** <claudia.lenk@pharmazie.uni-halle.de> — *Identification of herbivore-induced transcription factors involved in the terpene biosynthesis in maize*

P45 **Burkhard Schulz** <bschulz@purdue.edu> — *Interactions with growth media affect the efficacy of a potent brassinosteroid biosynthesis inhibitor in maize*

P46 **John Gray** <jgray5@utnet.utoledo.edu> — *Investigation of the role of a Divaricata type transcription factor in Zea mays.*

P47 **Alan Myers** <ammyers@iastate.edu> — *Maize opaque5 Encodes Monogalactosyldiacylglycerol Synthase and Specifically Affects $C_{18:3}$/$C_{18:2}$ Galactolipids Necessary for Amyloplast and Chloroplast Function*

P48 **Sylvia Morais de Sousa** <smsousa@cnpms.embrapa.br> — *Maize aldose reductase: A role in sugar-handling?*

P49 **Manmeet Singh** <manmeet.agri@gmail.com> — *Monitoring Ds Transposition in Soybean Genome*

P50 **Vijay Chaikam** <vchaikam@purdue.edu> — *Moving towards efficient, reliable and high throughput double haploid line development*

P51 **Jonathan Dennis** <dennis@bio.fsu.edu> — *Nucleosome distribution and promoter architecture at 400 genes in the maize genome*

P52 **Andrés Adolfo Estrada-Luna** <aestrada@ira.cinvestav.mx> — *Role of carbohydrate metabolism on the functioning of lodicules during the opening of staminate flowers in maize.*

P53 **Kai Ying** <yingk@iastate.edu> — *Structural Diversity among Maize Haplotypes*

P54 **Joseph Black** <josephbla@ufl.edu> — *Structure-function analysis of the maize NLR1 protein, a plant-specific nuclear-localized protein*

P55 **Antonino Malgioglio** <antonino.malgioglio@unimi.it> — *Study of a viviparous mutant impaired in the last phase of the ABA pathway*

P56 **Wenmin Qin** <qinw@iastate.edu> — *The cuticle of maize silks as a model system to define the pathway of hydrocarbon biosynthesis*

P57 **Rachel Mertz** <ram434@cornell.edu> — *The molecular genetic dissection of C4 traits in maize and Setaria viridis, model systems for C4 biology*

P58 **Sheila Juarez** <shejuacol@gmail.com> — *The stem as a dynamic carbohydrate reservoir in maize*

P59 **George Chuck** <georgechuck@berkeley.edu> — *Using the Corngrass1 gene to enhance the biofuel properties of crop plants*

P60 **Jun Zheng** <zhengjuncn@gmail.com> — *ZmCIPK9, a maize CBL-interacting protein is involved in the signaling pathway of potassium uptake in plant*

P61 **Thant Naing** <ice.negative@gmail.com> — *necrotic upper tips1 is responsible for proper water movement during the floral phase of development*

P62 **Piyusha Singh** <piyusha_singh@yahoo.com> — *Recent advances in Paramutation in Maize*

Cytogenetics

P63 **Dale Brunelle** <dale.brunelle@und.nodak.edu> — *Plant Phenotype Dosage Effects of Maize Simple B-A Translocations in a W22 Inbred Background*

P64 **Rashin Ghaffari** <rghaffari@plantbio.uga.edu> — *A New Variant of Maize Abnormal Chromosome 10 Confirms Independent Neocentromere Activity of Two Knob Repeats*

P65 **Robert Gaeta** <gaetar@missouri.edu> — *A spontaneous compensating translocation permits recovery of a telomere-truncated chromosome in maize*

P66 **Fangpu Han** <fphan@genetics.ac.cn> — *Centromere specific sequences change and centromere reactivation in newly formed chromosomes in maize*

P67 **Jihyun Moon** <moonj@berkeley.edu> — *Characterization of mtm99-14 and mtm99-25; meiotic mutants with defects in homologous pairing*

P68	**Morgan McCaw** <mccawm@missouri.edu>	*Competition of Different Sized Centromeres as an Examination of the Centromere Drive Hypothesis*
P69	**Debbie Figueroa** <Figueroa@bio.fsu.edu>	*Construction Of A Cytogenetic Map Of Maize In Oat Addition Lines Using Sorghum Bacterial Artificial Chromosomes (BACs) As Fluorescent Probes*
P70	**Rick Masonbrink** <remkv6@mail.missoui.edu>	*Endoreduplication of a Very Small Telomere-Truncated Minichromosome Derived From the B Chromosome*
P71	**Gaganpreet Sidhu** <gks27@cornell.edu>	*Genetic variation in the meiotic recombination pathway in maize*
P72	**Suketoshi Taba** <s.taba@cgiar.org>	*Identification and Characterization of a Population in situ of Perennial Teosinte found in Ziracuarétiro, Michoacán, Mexico*
P73	**Evelyn Hiatt** <EHiatt@kwc.edu>	*Identification of two mutations (smd3 and smd12) that abolish neocentromere activity at 180bp knob repeats but not TR1 repeats*
P74	**Rick Masonbrink** <remkv6@mail.missoui.edu>	*Increasing the copy number of minichromosomes derived from the B chromosome*
P75	**Tatiana Danilova** <tatianad@ksu.edu>	*Maize whole chromosome painting*
P76	**Liz Howe** <esh07@fsu.edu>	*Microscopic Analysis of Transgenic Maize Lines Expressing a Fluorescent Histone, H2B::mCherry*
P77	**Ashley Lough** <anl6d9@mizzou.edu>	*Mitochondrial DNA Sequences in the Nuclear Genomes of Diverse Maize Lines*
P78	**Choon Lin Tiang** <ct452@cornell.edu>	*Studying the meiotic telomere bouquet in maize using the pam1 mutant*
P79	**Han Zhang** <hzhang@plantbio.uga.edu>	*The evolution of Kinetochore size*

Development & Cell Biology

P80	**Josefine Nestler** <Nestler@uni-bonn.de>	*The root hair proteome of the maize inbred line B73*
P81	**Beth Thompson** <thompsonb@ecu.edu>	*fuzzy tassel encodes a DICER-LIKE1 protein and is required for vegetative and inflorescence development*
P82	**Fang Bai** <fangbai@ucsd.edu>	*A semi-dominant tassel seed on chromosome 7 of maize affects spikelet meristem determinacy in both the tassel and ear*
P83	**Michael Pautler** <pautler@cshl.edu>	*A targeted re-sequencing approach to identify the mutation underlying a fasciated ear mutant*
P84	**Brunie Burgos** <brunilis@uga.edu>	*A tissue-specific RNA interference strategy to study the role of Arabidopsis Minichromosome Instability 12 (MIS12)*
P85	**Edoardo Bertolini** <e.bertolini@sssup.it>	*Addressing the involvement of microRNAs in the reprogramming of leaf growth under drought stress in grasses*
P86	**Allison Phillips** <arphilli@stanford.edu>	*Analysis of stunter1, a Maize Mutant with Reduced Gametophyte Size and Maternal Effects on Seed Development*
P87	**Marie javelle** <mjavelle@vshl.edu>	*Analysis of small RNA-controlled gene networks in SAM function*
P88	**Sivanandan Chudalayandi** <csiva@iastate.edu>	*Analysis of the ZmHK1 gene family and its possible roles in leaf patterning*
P89	**Paula McSteen** <mcsteenp@missouri.edu>	*Auxin Evo-Devo: Genetic and genomic approaches to understanding the role of auxin in shoot development*
P90	**Alessandra Lanubile** <alessandra.lanubile@unicatt.it>	*Auxin content, cell size and endoreduplication level in the mutant defective endosperm-18*

P91	**Clinton Whipple** <whipple@byu.edu>	*Bract suppression evolved in the grass family following a series of duplication events that created the Tsh1 and Ntt gene lineages*
P92	**Thomas Hartwig** <thartwig@purdue.edu>	*Brassinosteroid control of sex determination in maize*
P93	**Wei Li** <wli@iastate.edu>	*Characterization and Cloning of tassels replace upper ear1 in Maize*
P94	**Steven Williams** <stevenkw84@gmail.com>	*Characterization of sterile tassel silky ear1, a PISTILATA-like mutant in maize*
P95	**Diane Janick-Buckner** <djb@truman.edu>	*Characterization of a New ROUGH SHEATH1 Mutant of Maize*
P96	**Jihyun Moon** <moonj@berkeley.edu>	*Characterization of novel anther mutants in maize*
P97	**Jun Huang** <junhuang@waksman.rutgers.edu>	*Characterization of the pollen-specific stk1 and stk2 genes in maize*
P98	**Yongxian Lu** <yxlu@stanford.edu>	*Clone and characterize the Tcb1 factor (s) that forms cross barrier between maize and teosinte*
P99	**Fang Yang** <fyang@cshl.edu>	*Cloning and characterization of a dominant phyllotaxy mutant (Abphyl2) in maize*
P100	**Ahmed Mazen** <amalmazny@gmail.com>	*Colonization of roots of the Egyptian corn (Zea mays), Sesame (Sesamum indicum) and cotton (Gossypium barbadense) plants by the endophytic basidiomycete Piriformospora indica promotes their growth and salinity tolerance*
P101	**Woong June Park** <parkwj@dku.edu>	*CsCl implicated a role for K^+ in the early growth of maize primary root*
P102	**Christy Gault** <cgault@ufl.edu>	*Deep sequencing of maize endosperm culture transcriptomes to understand the impact of the ROUGH ENDOSPERM3 splicing factor*
P103	**Michael Scanlon** <mjs298@cornell.edu>	*Development of the monocot sheathing leaf base: the role of NARROW SHEATH*
P104	**Gibum Yi** <gyi@iastate.edu>	*Duplicate naked endosperm genes encode ID domain transcription factors required for maize aleurone differentiation*
P105	**Irina Makarevitch** <imakarevitch01@hamline.edu>	*Effects of chromosome number variations on global gene expression*
P106	**Lindsay Moss-Taylor** <Ldmt005@iastate.edu>	*Functional analysis of the Hairy Sheath Frayed1 mutation in maize.*
P107	**Addie Thompson** <hall1116@umn.edu>	*Genetic Control of Natural Variation in Maize Meristem Architecture*
P108	**Gokhan Kir** <gkir@iastate.edu>	*Genetic and transgenic analysis of Brassinosteroids functions in maize*
P109	**Nathalie Bolduc** <nath.bolduc@gmail.com>	*Genome-wide analysis of KNOTTED1 binding sites using ChIP-seq*
P110	**Laurie Smith** <lgsmith@ucsd.edu>	*Identification of pan2 as a leucine-rich repeat receptor-like kinase promoting division asymmetry via a proteomic strategy*
P111	**Dale Aromdee** <daromdee@berkeley.edu>	*Identification of proteins that interact with the maize transcription factor KNOTTED1*
P112	**Katie Petsch** <petsch@cshl.edu>	*Inbred-specific modifiers of the maize ta-siRNA pathway*
P113	**K.V. Derkach** <katerina-d-d@yandex.ua>	*Influence of sucrose on in vitro callusogenesis of Lancaster inbred lines*

P114 **Alexander Goldshmidt** <goldshmi@cshl.edu>	*Investigation of molecular mechanisms controlling determinacy of the spikelet-pair meristem*
P115 **Norman Best** <nbbest@purdue.edu>	*Isolation of defective kernel mutants from an open-pollinated EMS population to identify maternal effects on seed developmental processes in maize*
P116 **Daniel Kirienko** <harkius@uwyo.edu>	*Live-cell imaging of ZmRAB2A1-YFP and ZmRAB1A-CFP with Golgi and ER during maize leaf cell expansion show both overlapping and independent patterns of expression*
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P118 **Katharina R. Haentzschel** <katharina.haentzschel@uni-hohenheim.de>	*Maize DH production - In search of effective colchicine-alternatives*
P119 **Gerry Neuffer** <gneuffer@gmail.com>	*New Dominant Mutants From EMS Mutagenesis*
P120 **Antony Chettoor** <chettoor@stanford.edu>	*New maize mutants defective in female gametophyte development*
P121 **Caroline Marcon** <caroline.marcon@zmbp.uni-tuebingen.de>	*Non-additive protein accumulation patterns in maize hybrids during embryo development*
P122 **Nathan Smith** <nathan@uoguelph.ca>	*Observations of an Ear - Heterosis in Potential kernel Number of a Hybrid Derived by the Mating of Sister Lines*
P123 **Michael Malahy** <mike.malahy@okstate.edu>	*Pattern of vegetative architectural development in green millet (Setaria viridis) under varied light regimes*
P124 **Toni Kazic** <toni@athe.rnet.missouri.edu>	*Patterns in Lesions and Leaves*
P125 **Setu Vora** <setu-vora@uiowa.edu>	*Phase change in normal and Cg1-overexpressing Brachypodium*
P126 **Ben Beydler** <benjamin-beydler@uiowa.edu>	*Phase-Specific Gene Expression Patterns in Maize Leaves*
P127 **George Pantoja** <georgepantoja@hotmail.com>	*Phenotypic Characterization and Mapping of a ragged leaf2 mutant of Zea mays*
P128 **John Fowler** <fowlerj@science.oregonstate.edu>	*Pollen Germination in Maize Does Not Require Changes in the Transcriptome*
P129 **Zara Tabi** <ztabi@ucsd.edu>	*Positional Cloning and Characterization of the Rotten Ear (Rte)/Truncated Inflorescence Development1 (Tid1) Gene in Maize*
P130 **Erica Unger-Wallace** <eunger@iastate.edu>	*The Sorghum bicolor ramosa1 locus impacts inflorescence architechture in transgenic maize and Setaria.*
P131 **Xin Meng** <xin.meng@pioneer.com>	*The FT-like ZCN8 gene functions as a floral activator and is involved in photoperiod sensitivity in maize*
P132 **Joshua Budka** <jsbudka@purdue.edu>	*The GRAS transkription factor Brachytic crinkled leaf1 controls plant height, leaf angle and leaf morphology in maize*
P133 **Cristian Forestan** <cristian.forestan@unipd.it>	*The PIN-FORMED family of auxin efflux carriers mediates auxin transport and accumulation shaping maize development*
P134 **Pinghua Li** <pl324@cornell.edu>	*The developmental dynamics of the maize leaf transcriptome as revealed through ultra high throughput sequencing*
P135 **Indrajit Kumar** <ikumar2@illinois.edu>	*The phytochrome interacting bHLH proteins affect photomorphogenic development in Maize*
P136 **Arco Brunner** <arco.brunner@uzh.ch>	*The quest for apomixis in Zea mays. Episode I: Raiders of the lost meiosis*

P137 **Byung-Ho Kang** <bkang@ufl.edu>
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P138 **Kin Lau** <lau3@purdue.edu>
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P139 **Amanda Wright** <amanda.wright@unt.edu>
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P140 **Anding Luo** <aluo@uwyo.edu>
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P141 **Brandi Sigmon** <bsigmon@iastate.edu>
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P142 **Marcela Dotto** <dotto@cshl.edu>
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P143 **Andreas Lausser** <andreas.lausser@biologie.uni-regensburg.de>
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P144 **Anokhee Patel** <anokhee@ufl.edu>
Bulk Segregant map positions of UniformMu seed mutants can identify co-segregating transposon insertions

P145 **Alyssa Bagadion** <bagadiona@ufl.edu>
Molecular mapping of a novel rough endosperm mutant locus to the short arm of chromosome 5

P146 **Zhenzhen Yang** <zzy5028@psu.edu>
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P147 **Kengo Morohashi** <morohashi.1@osu.edu>
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P148 **Lingling Yuan** <linglingyuan77@gmail.com>
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P149 **A. Mark Settles** <settles@ufl.edu>
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P150 **Daniel Vera** <dvera@bio.fsu.edu>
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P151 **Wei Chen** <wchen3@dow.com>
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P152 **Katja Machemer** <machemer.1@osu.edu>
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P153 **Zhijie Liu** <liuz@cshl.edu>
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P154 **yonglianzheng zheng** <zhyl@mail.hzau.edu.cn>
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P155 **Wei Chen** <wchen3@dow.com>
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P156 **Suchitra Chavan** <suchitra@uga.edu>
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P157 **Cody Postin** <postin1@illinois.edu>
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P159 **Prem Chourey** <pschourey@ifas.ufl.edu>	*Gene-dose dependent control of seed mass by endosperm-specific Miniature1 (Mn1)-encoded cell wall invertase (CWI), which also affects embryo mass and embryo sugar physiology*
P160 **Anupma Sharma** <anupma@hawaii.edu>	*Generating complete physical maps of maize centromeres*
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P162 **Farag Ibraheem** <fii100@uiuc.edu>	*Genetic variation for regulation of gene expression associated with N remobilization in maize*
P163 **Hong Yao** <yaoho@missouri.edu>	*Genome Dosage Effects on Heterosis and Gene Expression in Triploid Hybrids of Maize*
P164 **Sivanandan Chudalayandi** <csiva@iastate.edu>	*Global gene expression profiling in normal and interploidy endosperms*
P165 **Huabang Chen** <hbchen@sdau.edu.cn>	*Identification and Characterization of Maize MicroRNAs Involved in the Very Early Stage of Seed Germination*
P166 **Thomas Lübberstedt** <thomasl@iastate.edu>	*Identification and characterization of new brown midrib genes in maize*
P167 **Maria Casas** <casas.5@buckeyemail.osu.edu>	*Identification of the Gene Products Responsible for Maysin Biosynthesis in Maize*
P168 **Tiffany Langewisch** <tllhw9@mail.missouri.edu>	*Identifying Candidates for the Restorer-of-Fertility Gene Rf3 in Maize CMS-S*
P169 **Anne Sylvester** <annesyl@uwyo.edu>	*Illuminating Maize Biology: Investigating functional genomics in maize using tagged-fluorescent protein lines*
P170 **Thomas Lübberstedt** <thomasl@iastate.edu>	*Isolating Resistance Genes against Sugarcane Mosaic Virus in Maize*
P171 **Alessandra Lanubile** <alessandra.lanubile@unicatt.it>	*Maize transcriptome analysis upon Fusarium infection in relation with host and pathogen genotypes*
P172 **Ho Man Holly Tang** <tang@iastate.edu>	*Mapping and Functional Analysis of Brown-midrib2 (bm2) via RNA-seq*
P173 **Zuxin Zhang** <zuxinzhang@mail.hzau.edu.cn>	*MiRNA-mediated auxin signaling involved in initiation and elongation of crown roots of maize under submergence condition*
P174 **Mihai Miclaus** <mihai@waksman.rutgers.edu>	*Molecular Cloning of Opaque7*
P175 **Donald McCarty** <drm@ufl.edu>	*New mutants in accessible UniformMu seed stocks*
P176 **Jayanand Boddu** <jboddu@uiuc.edu>	*Nitrogen influenced transcriptome and metabolome analyses in maize*
P177 **Erica Unger-Wallace** <eunger@iastate.edu>	*Remobilizing Ds transposons for targeted mutagenesis in maize*
P178 **Wesley Barber** <barber4@illinois.edu>	*Repeat-associated small RNAs in the shoot apex vary among parents and following hybridization in maize*
P179 **Nathan Springer** <springer@umn.edu>	*Reshaping of the maize transcriptome by domestication*
P180 **Wei Wu** <wuwei@iastate.edu>	*SNP-based Bulked Segregant Analysis for the Speedy and Economical Mapping of Maize Mutants*
P181 **Robert Martienssen** <martiens@cshl.edu>	*Sequencing the maize methylome.*
P182 **Mingshu Huang** <mh728@cornell.edu>	*Structural and metabolic transitions of C3 rice and C4 maize leaf development and differentiation determined by a systems biology approach*

P183 **Erika Roach** <edr148@psu.edu> — *The Genetics of Sustainable Agriculture: Does Maize Gene Expression Respond to Soil Management?*

P184 **John Gray** <jgray5@utnet.utoledo.edu> — *The Grass Transcription Factor ORFeome (TFome) Project*

P185 **Ram Sharma** <rks73@cornell.edu> — *The degree of ascertainment bias in SSRs, SNPs and genotyping-by-sequencing (GBS)*

P186 **Ying Li** <yingli3@illinois.edu> — *The inheritance of small RNA expression patterns in Arabidopsis hybrids*

P187 **Nidia Sanchez-Leon** <nsanchez@ira.cinvestav.mx> — *The role of Pap and Pho1 genes in the maize phosphate starvation response*

P188 **Patrice G. Dubois** <patrice.g.dubois@gmail.com> — *The subfunctionalization of the maize phytochromes PhyB1 and PhyB2 is background-specific*

P189 **Rajandeep Sekhon** <rsekhon@glbrc.wisc.edu> — *Transcriptional and metabolic changes during induced senescence in maize*

P190 **Susann Uphoff** <uphoff2@illinois.edu> — *Transcriptome Analysis and Functional Annotation of Nitrogen-Related Genes in the Maize B73 Genome*

P191 **Jennifer Hawkins** <jennifer.hawkins@mail.wvu.edu> — *Variation for allelic gene expression in maize*

P192 **Jiabing Ji** <ji6@purdue.edu> — *slm1 (suppressor of lesion mimic-1) encodes an NBS-LRR protein required for the expression of the lesion mimic mutation les23 in maize (Zea mays L.)*

Education & Outreach

P193 **Eunsoo Choe** <echoe1@illinois.edu> — *Establishing in vivo maize doubled haploid production at the University of Illinois for education and research purposes*

P194 **James Burnette** <james.burnette@ucr.edu> — *Exploiting the extraordinary transposable element-mediated diversity of maize to illustrate key genetic concepts to undergraduates and high school students*

P195 **Edward Buckler** <esb33@cornell.edu> — *Maize: Mysteries of an Ancient Grain – a Traveling Museum Exhibition*

P196 **Anastasia Bodnar** <abodnar@gmail.com> — *MaizeResearch.org: Outreach for the maize genetics community*

P197 **Rale Gjuric** <rgjuric@ucdavis.edu> — *UC Davis Plant Breeding Academy*

P198 **John Gray** <jgray5@utnet.utoledo.edu> — *Using the maize TFome project to Foster the Integration of Research with Undergraduate Education (FIRE)*

Quantitative Genetics & Breeding

P199 **Meng Li** <ml598@cornell.edu> — *A genome-wide association study of 1495 transposable elements in Zea mays*

P200 **Joost van Heerwaarden** <jvanheerwaarden@ucdavis.edu> — *A genome-wide view of breeding history and selection in North American maize lines*

P201 **Jinliang Yang** <yangjl@iastate.edu> — *Bayesian-based GWAS: Identification of Loci Controlling Variation in Yield Component Traits in the Nested Association Mapping (NAM) Population*

P202 **Mehmet Ali Turkay** <turkay65@hotmail.com> — *Breeding Maize for Resistance to Pink Stem Borer (Sesamia nonagrioides Lefebvre)*

P203 **Christopher Schaefer** <schae317@umn.edu> — *Breeding Potential of Semi-dwarf Corn for Grain and Forage in the Northern U.S. Corn Belt*

P204	**Avinash Karn** <ak1876@truman.edu>	*Breeding Specialty Starch Maize Using Exotic Genetic Resources for Gene Discovery of Novel Alleles and Modifiers with materials generated from USDA-ARS GEM Project*
P205	**Marcelo Carena** <Marcelo.Carena@ndsu.edu>	*Breeding for Healthier Short-Season Maize*
P206	**Jode Edwards** <jode.edwards@ars.usda.gov>	*Changes in Plant Morphology in Recurrent Selection Programs in the Iowa Stiff Stalk Synthetic Population*
P207	**Zachary Lemmon** <zlemmon@wisc.edu>	*Characterization of Domestication QTL on Chromosome Five of Z. mays*
P208	**Hong Yao** <yaoho@missouri.edu>	*Comparison of Inbreeding Depression between Diploid and Tetraploid Maize*
P209	**Jason Cook** <cookjp@missouri.edu>	*Comprehensive Analysis of the Genetic Architecture of Maize Kernel Composition in NAM using Joint-Linkage Mapping and Genome Wide Association*
P210	**Jiahn Chou Guan** <guanjc@ufl.edu>	*Detection of quantitative trait loci (QTL) controlling the bouquet-ear trait in maize*
P211	**Huabang Chen** <hbchen@sdau.edu>	*Development of Functional Molecular Markers of SbeI and SbeIIb for High Amylose Maize Germplasm Line GEMS-0067*
P212	**Maria Magallanes-Lundback** <magalla1@msu.edu>	*Development of high through put analytical method for association maize and NAM of vitamins and essential amino acids in Maize seeds*
P213	**Zhiming Zhang** <zhangzm1979@yahoo.com.cn>	*Difference of Pb and Cd accumulation in 19 elite maize Inbred lines and application prospects*
P214	**Junping Chen** <junping.chen@ars.usda.gov>	*Dissection of High Temperature Tolerance Traits of Maize*
P215	**Timothy Beissinger** <beissinger@wisc.edu>	*Effect of allele frequency changes on the ability to detect loci of genetic importance in the Golden Glow maize population long term selection program*
P216	**Aaron Lorenz** <alorenz2@unl.edu>	*Effect of model and training population on genomic selection for multiple traits in maize*
P217	**Seth Murray** <sethmurray@tamu.edu>	*Effective Recombination in Plant Breeding and Linkage Mapping Populations: Testing Models and Mating Designs*
P218	**Jose Santa-Cruz** <jhsantac@ncsu.edu>	*Fine Mapping and Field Evaluation of a Quantitative Trait Locus conferring Resistance to Southern Corn Leaf Blight*
P219	**Michael Popelka** <mpopelka88@gmail.com>	*Functional Stay-green: Abiotic Stress Tolerance in Maize*
P220	**Ruairidh Sawers** <rsawers@langebio.cinvestav.mx>	*Functional mapping of maize phosphate responses*
P221	**Candice Gardner** <candice.gardner@ars.usda.gov>	*GEM – Meeting the Challenge of Maize Diversification by Capturing Useful Alleles and Traits from Exotic Germplasm*
P222	**Christine Lucas** <cjlucas@illinois.edu>	*Genetic Regulation of Grain Protein Concentration in Maize Following Long Term Selection*
P223	**Torbert Rocheford** <torbert@purdue.edu>	*Genetic Analysis of Maize Kernel Color Using Segregation, Bulked Segregant, and Nested Association Mapping Analyses*
P224	**Andrew Doust** <andrew.doust@okstate.edu>	*Genetic basis of plant architectural development in grasses – insights from foxtail millet*
P225	**Yuhe Liu** <yuheliu1@illinois.edu>	*Genetic mapping approaches to identifying N utilization genes in maize*

P226 **Peter Balint-Kurti** <peter_balintkurti@ncsu.edu>	*Genome-wide Association Mapping in the Maize NAM Population Permits Precise Identification of Loci Conditioning Southern Leaf Blight Resistance*
P227 **Feng Tian** <ft55@cornell.edu>	*Genome-wide association study of maize leaf using the second generation of maize HapMap*
P228 **Emily Combs** <comb0064@umn.edu>	*Genomewide Selection to Introgress Exotic Traits into Adapted North American Germplasm*
P229 **Steven Eichten** <eicht021@umn.edu>	*High resolution mapping of B73-Mo17 near isogenic lines*
P230 **Jenny Xiao** <jxiao@sequenom.com>	*High-throughput and Quantitative Genotyping Applications in Economically Important Crops*
P231 **Sara Larsson** <sjl65@cornell.edu>	*Hybrid Vigor in the Maize Nested Association Mapping Population*
P232 **Becky Weeks** <rlmauton@iastate.edu>	*Identification and mapping of branching modifiers in ramosa mutants*
P233 **David Hessel** <dhessel@iastate.edu>	*Identification of exotic alleles that confer native resistance to Western corn rootworm beetles*
P234 **Xianran Li** <lixr@ksu.edu>	*Identification of leaf trait related genes by genome-wide nested association mapping of maize leaf traits with HapMap and RNA-Seq SNPs*
P235 **Paul Zurek** <prz@duke.edu>	*Identification of natural variation in the root system architecture in the Nested Association Mapping (NAM) population.*
P236 **Alex Lipka** <ael54@cornell.edu>	*Identifying genes associated with provitamin A and vitamin E content in maize kernel through association mapping*
P237 **Ani A. Elias** <elias1@purdue.edu>	*Impact of weather and soil on the performance of hybrid maize in multi-environment trials*
P238 **Xingming Fan** <xingmingfan@163.com>	*Improving grain yield and yield components via backcross procedure*
P239 **Tristan Coram** <tecoram@dow.com>	*In-depth characterization of transgenic events carrying targeted insertions at the maize ZmIPK1 locus*
P240 **David Wills** <dwills@wisc.edu>	*Initial Characterization of zagl 1, a Target of Selection during Maize Domestication*
P241 **Jianming Yu** <jyu@ksu.edu>	*Integrating Biological Function Prediction, Rare Alleles Testing, and Functional Network Screening into Association Mapping for Flowering Time in Arabidopsis*
P242 **Emma Gachomo** <egachomo@purdue.edu>	*Integrating MAGIC and NAM to enrich the genetic network underlying the maize immune response*
P243 **Ann Meyer** <ameyer@uoguelph.ca>	*Investigating the genetic basis for naturally occurring primary root growth variation in Zea mays*
P244 **Jason Peiffer** <jap333@cornell.edu>	*Joint Linkage-Association Mapping of Maize Height and Related Traits*
P245 **Jeffery Gustin** <jgustin@ufl.edu>	*Kernel weight and composition quantitative trait loci (QTL) from single-kernel analysis*
P246 **Phillip Long** <pjlong@okstate.edu>	*Miami White Corn: Genetic Relationships Between Native American Landraces*
P247 **Loren Trimble** <latrimble@wisc.edu>	*Maize Domestication and Genetic Factors for Seedling Emergence*
P248 **Elizabeth Buescher** <ebuesche@purdue.edu>	*Maize interploidy hybridization barrier: a quantitative approach*

P249 **Luis Avila** <lavilabo@uoguelph.ca>	*Mapping and characterization of a flowering time mutant in Zea mays L.*
P250 **Xiaochun Sun** <sun54@illinois.edu>	*Model building in support of computer simulation to guide key decisions in plant breeding*
P251 **Erin Brinton** <ebrin001@ucr.edu>	*Molecular Responses of Maize to Submergence*
P252 **Christopher Topp** <chris.topp@duke.edu>	*Multidimensional analysis of root system architecture reveals differences in the Illinois High and Low Protein strains*
P253 **Marcelo Carena** <Marcelo.Carena@ndsu.edu>	*NDSU EarlyGEM: Increasing Genetic Diversity of Northern Corn Belt Hybrids with Tropical and Temperate Exotic Germplasms*
P254 **Travis Coleman** <colemant@uoguelph.ca>	*Nature of the Genetic Variation in an Elite Maize Breeding Cross*
P255 **Laura Shannon** <lshannon@wisc.edu>	*Pleiotropic effects of ZmGHD7 on flowering time and inflorescence structure in maize and teosinte*
P256 **Andrew Hauck** <ahauck@illinois.edu>	*Populations Derived From Elite Ex-PVP Commercial Inbreds: New Public Maize Genetics Resources*
P257 **Hanneke Witsenboer** <hwi@keygene.com>	*Power of a non-destructive high-throughput phenotype platform in unraveling drought and salt stress related traits*
P258 **Fei Lu** <fl262@cornell.edu>	*QTL Mapping of Chloroplast and Mitochondria Genome Copy Number in Maize*
P259 **Juan Marroquin** <juanjo@illinois.edu>	*QTL Mapping of Western Corn Rootworm (Diabrotica virgifera virgifera LaConte) Resistance in Maize*
P260 **Jason Morales** <jasonmorales@purdue.edu>	*QTL analysis for performance in elite Ex-PVP maize lines using a bi-parental mapping population*
P261 **Viridiana Silva Pérez** <silva.viridiana@colpos.mx>	*QTL associated with carotene content in maize leaves*
P262 **Jana Steinhoff** <jana.steinhoff@uni-hohenheim.de>	*QTL-Mapping based on joint multiple populations in a Maize Testcross Experiment*
P263 **Anastasia Bodnar** <abodnar@gmail.com>	*Response of transgene and native gene expression to selection for transgene levels*
P264 **Mark Jones** <jones.390@osu.edu>	*Responses of maize (Zea mays L.) near isogenic lines carrying Wsm1, Wsm2 and Wsm3 to three viruses in the Potyviridae*
P265 **Cathrine Ziyomo** <ziyomo001@umn.edu>	*Selective phenotyping to map QTL for drought and low soil-N stress tolerance in maize*
P266 **Nandita Limbu** <ndtlimbu@yahoo.com>	*Studies related to response of different growth stages of maize to waterlogging stress*
P267 **Cinta Romay** <mcr72@cornell.edu>	*Studying natural diversity on a region regulating flowering time on Chromosome 8*
P268 **Thomas Lubberstedt** <thomasl@iastate.edu>	*The Doubled Haploid Facility at Iowa State University*
P269 **Lewis Lukens** <llukens@uoguelph.ca>	*The Effect of Intermating on Hybrid Trait Variation and QTL Mapping in Maize (Zea mays L.)*
P270 **Rawikarn Pulam** <pulam1@illinois.edu>	*The Effect of Photoperiod Sensitivity and Variation in Nitrogen Utilization on Total Biomass Accumulation in Corn (Zea mays)*

Transposons & Epigenetics

P271 **Ann Armenia Ferguson** <armeniaa@msu.edu>
Mutator-like elements with tandem TIRs

P272 **Yubin Li** <yubin@waksman.rutgers.edu>
A single gene encodes the transposition functions of TED, an autonomous element of the Mutator superfamily

P273 **Diane Burgess** <dburgess@berkeley.edu>
Active MuDR elements modify but do not heritably reactivate silenced MuDR elements in maize

P274 **Chunguang Du** <duc@mail.montclair.edu>
Analysis of Ac/Ds transposons in the maize inbred lines B73 and W22

P275 **Nathanael Ellis** <nellis@uga.edu>
Characterizing histone modification dynamics in maize pericentromeres

P276 **Kameron Wittmeyer** <ktw5072@psu.edu>
Cloning ufo1 through transposon tagging

P277 **Charles Hunter** <ibe@ufl.edu>
Diversity and abundance of Mutator transposable elements in maize and teosinte inbreds: New insights into their evolution and behavior

P278 **Jianbo Zhang** <jzhang@iastate.edu>
Does Transposition-Mediated DNA Re-Replication Generate Segmental Duplications?

P279 **Cristian Forestan** <cristian.forestan@unipd.it>
Environmental stress-induced epiallele formation and inheritance in Zea mays: a multiple approach

P280 **Laura Vann** <laura.e.vann@gmail.com>
Evidence of introgression of a maize tb1 allele into populations of teosinte

P281 **William F. Sheridan** <bill.sheridan@und.edu>
Excision Frequencies of Eight Maize Ac Elements Located on the Short Arm of Chromosome 1

P282 **Dafang Wang** <dwang@iastate.edu>
Excision and Reinsertion of Ac Macrotransposons at the Maize p1 Locus

P283 **Matt Estep** <mcestep@gmail.com>
Genomic characterization for parasitic weeds of the genus Striga by sample sequence analysis

P284 **Mei Zhang** <zhangmei_2008_2006@126.com>
Identification of imprinting genes in maize endosperm through transcriptome sequencing

P285 **Ruijia (Kevin) Huang** <ruijia.huang@jacks.sdstate.edu>
Influence of genetic background on Ac/Ds transposition activity.

P286 **PoHao Wang** <puw116@psu.edu>
Maintenance of tissue-specific gene silencing and paramutation is associated with histone modification regulated by unstable factor for orange1 (ufo1) in maize

P287 **Mario Motto** <mario.motto@entecra.it>
Meiotically stable inheritance of natural variation for CG methylation in genetically identical maize plants

P288 **Xianyan Kuang** <xkuang@iastate.edu>
Modeling structure of Ac Transposase and Predicting Ds preferential insertion sites: a Bioinformatics approach to understanding Ac/Ds transposition in maize

P289 **Yubin Li** <yubin@waksman.rutgers.edu>
New reverse genetics resources for maize: facile production and efficient indexing using next-generation sequencing technology

P290 **Jonathan Gent** <gent@uga.edu>
On the role of RNA in centromere chromatin

P291 **Amanda Waters** <water157@umn.edu>
Profiling DNA methylation patterns in maize inbreds

P292 **Manjit Singh** <manjit.singh3@mcgill.ca>
Reproduction specific Argonaute genes in maize and barley and their role in transposon silencing

P293 **Joy-El Barbour** <joy-el.barbour@berkeley.edu>
The rmr2 locus encodes a novel protein required for small RNA biogenesis and paramutation

P294 **Dongyan Zhao** <zhaodon4@msu.edu>	*The insertion polymorphism of Mutator-like elements (MULEs) and their influence on gene expression upon long-term selection in maize*
P295 **Allison Barbaglia** <ambarbag@oakland.edu>	*Tissue Specific Alternative Splicing Expression of Helitron-captured Genes in Maize*
P296 **Thomas Peterson** <thomasp@iastate.edu>	*Transposon-Induced Chromosome Engineering in Plant Genomes*
P297 **Steven Eichten** <eicht021@umn.edu>	*Variation in genome-wide DNA methylation patterns among maize inbreds*

Plenary Talk Abstracts

Plenary 1

Grain Yield: the known knowns and the known unknowns…

(presented by Elizabeth Lee <lizlee@uoguelph.ca>)
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Grain yield is a highly complex and poorly understood trait. Yet despite this knowledge gap, maize breeders have increased grain yield 7-fold during the past 7 decades. While past success can be a good indicator of future successes, several groups have suggested that this rate of genetic improvement is becoming difficult to maintain and that the gains are coming at greater costs in terms of resources. Why? One possible cause of this is that the "biological weaknesses" that have been inadvertently targeted by plant breeders are no longer "biological weaknesses" in the elite germplasm pool. What will be the next "biological weakness" to target? Unfortunately this is where the "poorly understood" aspect of grain yield as a trait becomes a problem. In short, it is probably time to focus our collective efforts on understanding the biology underlying grain yield in maize. And, that leads us to what are the "known knowns" of grain yield, and what are the "known unknowns" of grain yield? What is grain yield? Conceptually grain yield is pretty simple. The crop captures CO2 and using energy from the sun stores this carbon in the form of starch, roughly half of which is stored in the grain. The more CO2 captured and stored in this manner on a per area basis, the greater the grain yield. From this over simplified concept of grain yield we will explore the developmental progression in the maize plant of 3 complex physiological mechanisms involved in grain yield: light capture and distribution, photosynthesis, and sink formation. We will examine key developmental time points that impact potential yield and how these 3 physiological mechanisms have changed during the past 7 decades primarily in response to increasing plant densities. Finally we will explore several of the known unknowns - processes, mechanisms and phenomena that "should be", "appear to be", or "are obviously" important.

Funding acknowledgement: NSERC, OMAFRA, and Grain Farmers of Ontario

Plenary 2

Engineering centromeres to produce haploid plants

(presented by Simon Chan <srchan@ucdavis.edu>)
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Centromeres are essential for chromosome segregation during mitosis and meiosis. Therefore, it is paradoxical that their DNA sequences and the sequences of kinetochore proteins are very fast evolving. This observation has led to the suggestion that centromere differences can cause hybrid infertility when two different species are crossed.

The centromere-specific histone CENH3 replaces H3 in centromeric nucleosomes, and is essential for kinetochore function. We have found that large-scale chromosome missegregation occurs when Arabidopsis thaliana plants expressed altered CENH3 proteins are crossed to wild type. Chromosomes that contain mutant CENH3 are completely eliminated in up to 50% of F1 progeny, yielding haploid plants with chromosomes from only their wild type parent. Either the maternal or paternal chromosomes may be eliminated depending on the direction of the cross. Chromosome competition after fertilization is a subtle assay for centromere defects, because some of the CENH3 mutants that cause missegregation in a cross are phenotypically indistinguishable from wild type.

Haploid plants are easily converted back to diploids, allowing Arabidopsis geneticists to instantly produce true-breeding varieties from heterozygous parents. Our research may greatly accelerate plant breeding if genome elimination can be re-created in crops. We also show that changes in a single centromere protein can cause chromosome missegregation when two parental genomes are mixed after fertilization. This may indicate how rapid centromere evolution can create species barriers.

Funding acknowledgement: National Science Foundation (NSF)

Plenary 3

Good science in maize genetics: Is serendipity involved?

(presented by Ronald Phillips <phill005@umn.edu>)
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Some scientists are afraid to do an experiment because they worry that the results will not be of sufficient merit. This presentation is intended to encourage especially the young scientist to ask an important question and then follow the leads that might come from serendipity. Examples of serendipity in my own research include: 1) An initial goal of developing maize strains with various knob combinations led to the identification of an apparent NOR duplication, providing the material for an unanticipated objective to locate the ribosomal RNA genes of maize to chromosome 6, and to estimate the number of gene copies at an unexpected 8000. 2) A goal of understanding the genetics of a rough-textured kernel that was shown to be due to a brittle-mutable allele controlled by the Spm/En system; interestingly the material was then used to clone the Brittle-1 gene. 3) The technology for the regeneration of whole corn plants from tissue culture involved the use of the proper tissue, culture medium, and genotype. Unexpected was the use of this technology for the genetic engineering of maize where today 24% of global maize is biotech. 4) Crosses of maize and oats were made to produce haploid oat plants. About one-third of the embryo-rescued progeny had the haploid number of oat chromosomes and, unexpectedly, one or more maize chromosomes. This led to a high-throughput method of mapping maize DNA sequences, and possibly may lead to the transfer of C4 traits to a C3 plant. 5) In attempting to clone a QTL for early flowering, it was discovered that the pertinent sequence was, unexpectedly, non-coding and probably interacted with an apetula-like gene 70 kb upstream. So, start your experiments on an important question but be sure to follow serendipitous leads as an effective way to make new discoveries.

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

Plenary 4

Epigenetic control of seed development in Arabidopsis

(presented by Ueli Grossniklaus <grossnik@botinst.uzh.ch>)
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In flowering plants, seed development is initiated by double fertilization, where two pairs of gametes fuse to form the embryo and endosperm, respectively. Over the last decade, it has become apparent that epigenetic processes play a crucial role in seed development. However, it is not clear to what extent maternal contributions are involved in early embryogenesis. Defining the contributions and interactions of paternal and maternal genomes during embryo development is critical to understand the fundamental processes involved in heterosis, hybrid sterility, and reproductive isolation. We have used the model plant *Arabidopsis thaliana* to determine the parental contributions and their regulation during early embryogenesis. To this aim we combined deep-sequencing-based, allele-specific RNA profiling and genetic analyses. At the 2-4 cell stage there is a strong, genome-wide dominance of maternal transcripts, although transcripts are contributed by both parental genomes. At the globular stage the relative paternal contribution is higher, largely due to a gradual activation of the paternal genome. We identified two antagonistic maternal pathways that control these parental contributions. Paternal alleles are initially down-regulated by the chromatin siRNA pathway, linked to DNA and histone methylation, while transcriptional activation requires the maternal activity of the histone chaperone complex CAF1. We propose that the interplay between activating and repressing maternal factors defines the distinct timing and kinetics of paternal genome activation for each locus. In summary, our results define novel maternal epigenetic pathways controlling the parental contributions in plant embryos, which are distinct from those regulating genomic imprinting.

Funding acknowledgement: SNF, RRF, CNRS, IRD, ANR, HHMI, European Union.

Short Talk Abstracts

T1

A Transposon Insertion was the Causative Mutation in the Maize Domestication Gene *tb1*

(presented by Anthony Studer <studer@wisc.edu>)
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Maize and its wild progenitor, teosinte, show a striking difference in plant and ear morphology that is partially governed by a regulatory element or "control region" in the *teosinte branched1* (*tb1*) gene. This control region is located ~58.8 kb upstream of the transcription start site, and causes an approximately two-fold increase in *tb1* expression in maize compared to teosinte. We performed recombination mapping, using teosinte chromosomal segments containing the control region introgressed into a maize inbred background, to dissect the genetic changes underlying the differences between maize and teosinte. We show that the *tb1* control region is complex, having two components with independent effects on the plant phenotypes that distinguish maize and teosinte. The common maize haplotype for the control region possesses two transposable element insertions that are not found in the common teosinte haplotypes. Using transient expression assays, we show that one of these transposon insertions acts as an enhancer of gene expression, consistent with the higher level of *tb1* expression seen in maize. Molecular dating indicates that the transposon insertions predate maize domestication by more than 10,000 years, indicating that selection acted on standing variation rather than new mutation. Our results highlight how transposons can contribute to evolution and domestication through alterations in gene regulation.

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

T2

Large-scale epigenetic reprogramming during maize seed development

(presented by Jose Gutierrez-Marcos <j.f.gutierrez-marcos@warwick.ac.uk>)
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In flowering plants both products of double fertilization undergo very different fates despite sharing the same genetic make up. These differences have been attributed to epigenetic changes that take place during or after fertilization. Such epigenetic reprogramming predominantly affects the endosperm and studies have shown the occurrence of widespread demethylation and an abundance of siRNAs of maternal origin in this organ. However, the significance or implications of this large-scale epigenetic reprogramming during endosperm development remains unclear.
Here we describe the genome-wide expression profiling of siRNA, mRNA, and DNA methylation using next generation sequencing and computational methods. We show evidence of maternally derived siRNAs that direct DNA methylation to discrete loci of the maize genome. Intriguingly, this methylation is depleted from the maternally inherited genome thus contributing to the establishment of differentially methylated regions (DMRs) between parental alleles in the endosperm. This epigenetic asymmetry is altered in mutants affecting the siRNA pathway. Further we show evidence for endosperm-specific siRNAs that play a pivotal role in directing DNA methylation to the developing embryo. We postulate that this epigenetic reprogramming in the ephemeral endosperm tissues takes place as a means of influencing the fate of the offspring.

Funding acknowledgement: BBSRC & The Royal Society

T3

Coordinate regulation of multiple targets of epigenetic silencing during maize development

(presented by Hong Li <hongli@berkeley.edu>)
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Plants have evolved a particularly diverse set of small RNA silencing pathways, which allows them to cope with a variety of intrinsic and environmental changes. Mounting evidences show that these small RNA pathways are interconnected. Our previous work has shown that MuDR transposon silencing is alleviated during the juvenile-to-adult phase transition. This transient relaxation coincides with down-regulation of lbl1 (the maize homolog of SGS3), and up-regulation of a target of the tasiRNA pathway, arf3a. Thus, our results suggest that both transposon silencing and tasiRNA pathway are coordinated during vegetative phase transition via changes in lbl1 expression. SGS3 is also required for nat-siRNA production in Arabidopsis. nat-siRNAs derive from dsRNA formed by convergent transcription of cis-antisense gene pairs. To find conserved cis-antisense genes in maize, we started with a published list of pairs of such genes in rice that have corresponding small RNAs in the overlapping regions. We then identified those pairs of genes that are highly conserved in five grass species, including maize. Given the high degree of insertional polymorphism in the grasses, such a high level of retention of cis-antisense configuration suggests selection in favor of this orientation. In order to identify the role of lb1 in regulation of these genes, we examined their expression level wild type and lbl1 mutant siblings. In nine of eleven cases examined, at least one of the genes in each pair was dramatically up-regulated in the mutant. In addition, in five cases examined to date, expression of these genes was also up-regulated in transition leaves. These results show that transposon taming and endogenous gene silencing (the tasi-RNA and nat-siRNA pathways) can be co-regulated, and that epigenetic reprogramming may occur on a broad scale during vegetative phase transition.

T4

Identification and Characterization of Gene Coexpression Networks in Maize Development

(presented by Gregory Downs <gdowns@uoguelph.ca>)
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By varying transcriptional profiles both spatially and temporally, plants create a variety of structures at different developmental stages. To obtain a genome-wide view of transcriptional circuits in the agriculturally significant crop species *Zea mays*, we examined transcript abundance data from various developmental stages (from embryo to senescence) for each gene across a number of time points and across a number of tissues and identified modules of genes that share coexpression patterns. Tissue samples were collected in triplicate from 50 different tissue types at different developmental stages, including leaf (nine stages), root (eight stages), tassel (eight stages), stalk, cob, silk, husk, embryo, and endosperm (three to five stages each). We examined the expression profile of each gene across the 50 tissue types on a custom Affymetrix microarray, and used hierarchical clustering of gene interconnectedness and topological overlap to form modules. Modules were correlated with tissues and examined for enrichment of Gene Ontology (GO) terms. We found sixty-three modules, ranging in size from 36 to 5103 genes (mean 553, median 98). Modules revealed remarkable patterns of tissue-specific expression, with few modules exhibiting a significant correlation with more than one tissue type. There was little relationship between the complexity of sampled tissue and the number of modules significantly correlated with the tissue, and developmentally similar tissues (e.g. husk and leaf) rarely shared modules. GO terms illustrate the biological significance of a number of modules. Our results suggest that tissues tend to have specific coexpression profiles, and these profiles represent biological functions important for maize development.

T5

The two different genomes within maize: differences in post-WGD gene loss, PAV, expression, and classical maize genes

(presented by James Schnable <jschnable@berkeley.edu>)
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Maize is ideally placed for investigating of the evolutionary consequences of whole genome duplication. The lineage leading to maize, teosinte, and tripsacum experienced a whole genome duplication between 5 and 12 million years ago. By comparing to a related but not duplicated outgroup species, sorghum, it is possible to reconstruct two orthologous copies of each sorghum chromosome within the maize genome. For all reconstructed chromosome pairs, one copy has consistently lost more genes syntenically conserved between rice and sorghum than the other. We grouped the chromosome copies that have experienced less total gene loss across their entire lengths following whole genome duplication into one subgenome, maize1, and the chromosome copies that experienced more gene loss into maize2. Maize1 gene copies tend to be expressed at higher levels than their duplicates on maize2. Maize2 gene copies are more likely to show presence absence variation between diverse inbreds providing evidence biased gene loss is ongoing in maize today. We propose that genome dominance, a phenomenon observed in recent and syntenic allopolyploids such as cotton and tetrapogon, may explain biased gene loss following whole genome duplication. Deletion of the less expressed member of a gene pair results in a smaller changes in total expression and may be less likely to impact fitness. Genes cloned as a result of visible mutant phenotypes are disproportionately located in the maize1 subgenome suggesting the mutation of an equivalent maize2 gene may be less likely to produce phenotypes drastic enough to be spotted by geneticists, and lending support to our overall model.

Funding acknowledgement: National Science Foundation (NSF)

T6

Genome-wide effects of domestication and improvement in landraces and modern maize

(presented by Matthew Hufford <mbhufford@ucdavis.edu>)
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Much progress has been made toward unraveling the genetic architecture of maize evolution through quantitative trait and population genetic analyses. However, these approaches have not yet employed full-genome resequencing and are therefore limited in scope. We sequenced 75 wild relative, landrace, and modern improved line genomes to an average depth of more than 5x yielding a dataset of over 21 million high-quality single nucleotide polymorphisms. Our comprehensive sampling allowed for an evaluation of the distinct bouts of selection during initial maize domestication and subsequent improvement. Genome-wide data provided evidence for much stronger selection during domestication than improvement, with a more pronounced bottleneck on diversity observed between the wild ancestor (*Zea mays* ssp. *parviglumis*) and landraces than between landraces and improved lines. Additionally, a paucity of fixed differences between genomes of maize and *Z. mays* ssp. *parviglumis* and signals of introgression from wild relatives into maize imply that standing variation from wild taxa has played a vital role during domestication and later adaptation to new environments during expansion. Our scan for selection identified several genes previously implicated in maize domestication and improvement but also generated a number of new, uncharacterized candidates with compelling functional annotations based on orthology to rice and *Arabidopsis thaliana*. Expression analyses based on long oligo hybridization arrays revealed higher and less varied expression in domestication candidates relative to non-candidates. Published expression data from a subset of crosses between maize inbred lines were also utilized to assess patterns of dominance in our candidate genes. Data on the likelihood of selection across the maize genome and a list of putative domestication and improvement candidates will be made publicly available and serve as valuable resources for future evolutionary studies and continued maize breeding efforts.

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

T7

SUN (Sad1p/Unc-84) Domain Genes Of Maize: Evidence for a Small Gene Family of Two Ancient Isoforms

(presented by Hank Bass <bass@bio.fsu.edu>)
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Meiosis requires dynamic control of chromosome topology and nuclear architecture in order to reduce the genome from the diploid to the haploid state. In many eukaryotes, the attachment and clustering of telomeres on the nuclear envelope (NE) during early meiotic prophase facilitates homologous chromosome pairing and recombination. Relatively little is known about the proteins in the plant NE, despite the expectation that they are conserved amongst eukaryotes. In maize, the desynaptic (dy) mutation leads to reduced recombination and the formation of univalents, resulting in a semi-sterile phenotype. We previously showed, using 3D telomere FISH, that the mutation also results in a precocious telomere-NE detachment during mid-prophase. We have used B-A translocation and BSA mapping to localize dy on the long arm of chromosome 3, where we have found a candidate gene with homology to the SUN-domain protein gene family. We have named this gene ZmSUN3, and found that it belongs to small gene family (ZmSUN1 - ZmSUN5) encoding SUN domain proteins. The maize SUN genes fall into two structural classes based on protein secondary structure and phylogenetic analysis. One of these has a canonical C-terminal SUN domain, whereas the other has a uniquely-positioned SUN domain (middle of the protein) plus three transmembrane domains. We call these the CSSD and PM3 types, respectively. These structural variants appear common to other plant species, including moss and Arabidopsis. Immunolocalization studies revealed a unique pattern of nuclear envelope staining during meitoic prophase for the CSSD class, implicating their role in post-bouquet chromosome interlock resolution and recombination control.

Funding acknowledgement: National Science Foundation (NSF)

T8

Mitochondrial DNA Sequences in the Nuclear Genomes of Diverse Maize Lines

(presented by Ashley Lough <anl6d9@mizzou.edu>)
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The incorporation of mitochondrial DNA (mtDNA) into nuclear genomes continues to occur in flowering plants. We have examined the mtDNA insertion sites within maize nuclear chromosomes (NUMTs) via in fluorescence *in situ* hybridization (Lough et al. Genetics 2008). MtDNA from the NB genome cloned into cosmids was used as probes onto metaphase chromosomes from root tips. The size and distribution of mtDNA insertions varies among maize inbred lines, accounting for some of the chromosomal diversity within maize. In B73, one large insertion on chromosome 9L was shown to contain a majority of the NB mitochondrial genome (Lough et al. Genetics 2008). An examination of a subset of the "diversity lines" of maize (Yu et al. Genetics 2008) is being conducted. Large NUMTs similar to the one reported in B73 have been found on 9L in Oh7B, a non-stiff stalk line, and HP301, a popcorn line. A much smaller insertion of mtDNA, containing only a small part of the mitochondrial genome, exists at this location in many other inbreds. We hypothesized that the small amounts of mtDNA are remnants of an older insertion created early in maize evolution. We have examined a total of 10 inbred lines and two teosinte subspecies (*Zea mays* ssp. *parviglumis* and *mexicana*) for the presence of the pieces of mtDNA shared by the all the previously analyzed lines. We also assessed these lines for the presence of two non-NB mtDNA pieces (2.4 kb and 3.3 kb long) found within overlapping BACs at the B73 9L location. These non-NB mtDNA pieces match the sequence of other Zea mitochondrial genomes, including *Zea mays* ssp. *parviglumis*.

Funding acknowledgement: National Science Foundation (NSF), University of Missouri Life Sciences Fellowship, University of Missouri Life Sciences Undergraduate Research Opportunity Program

T9

Analyses of shoot meristem organization during maize embryo development

(presented by Elizabeth Takacs <emt32@cornell.edu>)
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Maize shoot development is wholly dependent on stem cell maintenance and organ initiation, the two fundamental functions of the shoot apical meristem (SAM) that arise during the transition stage of early embryo development. Organization of the SAM is roughly coincident with development of the prominent hood of the scutellum, the first lateral organ initiated during grass embryogenesis. We are interested in deciphering the developmental mechanisms of shoot meristem formation and function. Pairing laser-capture microdissection (LCM) of proembryo and transition stage embryos with RNA-sequencing, we identified genes transcribed during the onset of SAM organization and during scutellum initiation in maize. 367 gene transcripts are detected in the transition-staged embryo that are absent in the pre-meristematic proembryo, which represent a cadre of transcripts expressed in the organizing SAM and/or the initiating scutellum. Enriched for genes of unknown function (110), transcription factors (84), and signaling genes (24), these differentially expressed transcripts also include known regulators of stem cell function (i.e. Class I KNOX genes and WOX genes) and lateral organ initiation (i.e. YABBY, LOB, NAC, and GRF genes). We are further investigating the expression pattern of interesting candidate genes implicated to function during meristem ontogeny and or scutellum development, including the transcription factor *yabby14* (*yb14*) and *tapetum determinant1* (*tpd1*), a signaling peptide necessary for cell fate determination in the tapetum. Transcripts of *yb14* and *tpd1* accumulate in the initiating scutellum during the transition phase, and thus are excellent developmental markers for investigating the ontogeny and homology of this grass-specific lateral organ.

Funding acknowledgement: National Science Foundation (NSF)

T10

VP8 and BE1, two distinct membrane-localized proteins, regulate embryo development and lateral organ numbers

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Viviparous8 (*vp8*) and *Big embryo1* (*be1*) genes have key roles in determining the number of lateral organs produced in maize. In contrast to several other genes implicated in lateral organ development, these two loci also regulate embryo development. Whereas *vp8* mutants severely perturbed embryo development in the W22 inbred, *be1* mutants develop viable embryos with more specific and subtle changes. The *be1* mutant produces the greater size of embryo than wild type with significant enlargement of the scutellum during seed development. The *vp8* and *be1* plants have pleiotropic phenotypes that have some common features, including increased numbers of leaves and roots. However, each mutant plant has distinct characteristics as well, including effects on timing of phase change and flowering. As we have previously reported, *Vp8* encodes putative membrane-localized peptidase (Suzuki et al., 2008). Subcellular localization using a GFP fusion protein indicated that VP8 is localized to ER. We have recently identified the *be1* gene by positional cloning and show that it encodes a membrane localized protein unrelated to VP8. The biological roles of *Be1* orthologs in Arabidopsis and rice are thus far undetermined. Thus, the identification of maize *Be1* highlights a novel signaling mechanism involved in regulation of seed and plant architecture. In particular, the predicted biochemical function and sub-cellular localization of BE1 indicate that specific secretion pathways play a critical role in determination of embryo size and lateral organ number.

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

T11

A negative regulator and duplicate IDD transcription factors control aleurone cell differentiation

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Aleurone cells have key biological functions as well as various human benefits. Aleurone cell fate specification is a dynamic process requiring positional cues throughout endosperm development. The *dek1* (*defective kernel1*) gene encodes a membrane localized calpain protease required for aleurone specification. Downstream substrates for DEK1 are not known. Here we report the analysis of two new genetic functions in the aleurone cell specification system. The *thick1* (*thk1*) gene is a negative regulator of aleurone fate because the loss-of-function mutant has an aleurone layer 4-6 cells deep, compared to the single layer in wild type. The *thk1* mutant is epistatic to *dek1*. *thk1* mutant sectors in a *dek1* mutant background (i.e. the sectors were double mutant) showed the thick aleurone phenotype suggesting *thk1* functions downstream of *dek1*. The mutant is associated with a deletion of approximately 2.3 megabases on chromosome 1. Experiments are ongoing to identify the specific gene within the deleted region responsible for the mutant phenotype. A second mutant, *naked endosperm* (*nkd*), shows defects in the specification of aleurone cell layers and in aleurone cell differentiation. Mutants contain approximately 3 peripheral cell layers instead of one and mutant cells show sporadic marker gene expression and lack the dense granular cytoplasm of normal aleurone cells. Thus, dual functions appear affected. Mutant plants are morphologically normal but show retarded development and delayed flowering. This mutant shows a 15:1 F2 segregation ratio suggesting it identifies duplicate gene functions. The mutant was mapped to syntenic genes encoding duplicate IDD zinc finger transcription factors. A new *Ds*-containing allele of the *nkd1/IDDveg9* gene failed to complement the original mutant, confirming the gene's identity, and showed unexpected segregation ratios suggesting a dosage effect. Compensatory transcript expression changes in single mutants suggest a feedback relationship between the loci. Further analysis is underway.

Funding acknowledgement: United States Department of Agriculture (USDA)

T12

New mutants in accessible UniformMu seed stocks

(presented by Donald McCarty <drm@ufl.edu>)
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The UniformMu project is creating a public reverse genetics resource for maize that provides ready access to knockout mutations in thousands of maize genes. Thus far, over 24,000 unique germinal Mu insertions have been mapped in a set of 5,400 UniformMu F3 stocks using NexGen sequencing methods. We expect to map up to 40,000 insertions in 8,000 F3 lines by Summer 2011. The precisely mapped, germinal insertions are searchable online at MaizeGDB by BLAST and genome browser tools. This site also links users directly to high-quality, sustainable seed stocks distributed without charge by the Maize Genetics Stock Center. Annotation of insertions using a filtered AGP v2 gene set indicates that at least 79% of the Mu insertions are in genes (64%) or promoters (500 bp, 15%). The collection contains hits in at least 12,000 maize genes including 4,300 genes that have two or more insertion alleles. Multiple insertion alleles are especially valuable to researchers for confirming gene functions. Mu insertion frequencies genome-wide were highly correlated with gene density (R^2= 0.9); whereas the correlation with recombination frequency was lower by comparison (R^2 = 0.65). Statistical analysis of insertion clustering in the genome indicates that Mu preferentially targets compact regions (<1 kpb) that frequently are associated with 5'-ends of genes. A mixed Poisson model of Mu insertion frequency predicts two classes of Mu targets that differ by 4-fold in average frequency of Mu insertions indicating absence of strong "hotspots" in UniformMu. Features that distinguish the low and high frequency classes of insertion targets are under investigation. Our results confirm that Mu is a highly effective mutagen for comprehensive functional analysis of the maize genome. The UniformMu resource has increased 10-fold in the last year. Check now for new Mu inserts in genes of interest.

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T13

Use of Illumina Sequencing to Identify Transposon Insertions Underlying Mutant Phenotypes in High-Copy Mutator Lines of Maize

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The utility of high-copy transposons for forward genetics has been hampered by the difficulty of identifying the specific insertions responsible for phenotypes of interest. We developed a method that substantially increases the throughput of identifying the causal insertions underlying interesting phenotypes in high-copy Mutator (Mu) lines in maize. The approach uses the Illumina platform to obtain sequences flanking all Mu elements in pooled, barcoded DNA samples. DNA is prepared for sequencing by shearing, adapter ligation, and hybrid-selection of fragments harboring Mu terminal sequences. The method yields clusters of sequence reads that tile ~400 base pairs flanking each side of each heritable insertion. A data analysis pipeline calculates insertion sites and compares insertions among individuals of known genotype to identify those that are linked to the mutation of interest. The rate-limiting step in this process is the development of the two-generation pedigree from which the DNAs are obtained.
We developed Mu-Illumina to use in conjunction with our collection of non-photosynthetic maize mutants, the Photosynthetic Mutant Library (PML), for the purpose of saturation mutagenesis of chloroplast biogenesis and photosynthesis. Many mutants in the PML collection have characterized chloroplast protein and RNA defects that provide considerable insight into gene function. In approximately 1.5 person-years we have "cloned" 28 causal insertions with this method, with a success rate of ~90%. This has allowed us (i) to assign functions to genes that, although conserved, had not previously been studied in any organism; and (ii) to discover maize-specific aspects of gene function for orthologs of genes that had been studied in Arabidopsis. This method could be adapted for use with other transposons, and greatly enhances the utility of high copy transposons for saturation mutagenesis of complex biological phenomena. A byproduct of the approach is the identification of numerous heritable insertions that are unrelated to the targeted phenotype. We are making such bystander insertions available for community use via a search interface at http://teosinte.uoregon.edu/mu-illumina/.

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T14

Peptide-Driven Gene Models and Protein Atlas of the Developing Maize Seed

(presented by Justin Walley <jwalley@ucsd.edu>)
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A fundamental goal of genome science is to discover all of the protein-coding genes. Complete and accurately annotated proteomes provide the foundation for studies of systems biology and molecular evolution, as well as for hypothesis-driven research. Recent progress in proteogenomics (using proteomic information to annotate the genome) has established it as a data driven method that complements nucleotide-based annotation strategies. Genome-wide, quantitative proteomics also makes possible the creation of a protein atlas that reveals the anatomical and subcellular distribution of the proteome. We will describe a protein atlas of the developing maize seed including differences in protein and phosphorylation levels between stages of development and between tissues. Proteins from more than 12,000 genes were identified and measured. These results are based on 86 million tandem mass spectra of 137,000 non-modified peptides and 23,000 phosphopeptides. Several proteins with known distributions were used to validate our data. All of the endosperm proteins known to participate in starch biosynthesis were measured and their sites of phosphorylation were mapped, creating the possibility of making site-directed mutations that regulate starch synthase assembly. We have used the mass spectra to discover new protein coding genes and to refine existing gene models that suffer from one or more problems (e.g., wrong frame, missing exons, or incorrect exon borders). These results will enhance genome-enabled maize research and breeding by increasing the completeness and accuracy of maize genome annotation. Investigations of maize physiology will benefit from the maize protein atlas.

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T15

The maize leaf: an ideal tool to study cell division and cell expansion, the processes driving growth

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The cells within a maize leaf can be classified according to their characteristics along the leaf length 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. This spatial gradient of growth processes allows specific enrichment of dividing, expanding or mature cells in samples taken across the growing leaf. The growth of the leaf can be monitored by the leaf elongation rate and the contributions of cell division or cell expansion can be quantified by a kinematic analysis. In this way we assessed the effects of several adverse conditions, such as mild drought and cold nights, and genetic perturbations on leaf development and more specifically on the processes of cell division and cell expansion. We integrated this cellular analysis with multiple systems-wide approaches such as transcriptome, metabolome and more recently hormonome profiling in an attempt to link the cellular responses to the underlying molecular mechanisms. The latest data will be presented together with the progress of the development of tools for maize transgenic and systems biology research.

T16

Reinventing MaizeGDB

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The Maize Database (MaizeDB → MaizeGDB) turns 20 this year, and such a significant milestone must be celebrated! With the release of the B73 reference sequence and more sequenced genomes on the way, the maize community needs to address various opportunities and challenges. MaizeGDB, the community's Model Organism Database is in an excellent position to address evolving needs by deploying tools that will allow researchers to make use of multiple genome sequences as well as large-scale phenotypic associations. To prepare for both current and future needs of the community, the MaizeGDB team is planning a complete interface redesign to keep the resource relevant and to allow for expansion in desired directions while continuing to update the data as usual over time. The redesign will be both cosmetic and functional. The overall goal of the redesign is to create a clean modern interface with improved user interaction while expanding the overall functionality of MaizeGDB. Cosmetically, we will modernize the appearance and simplify page organization and navigation. Functionally, we will put particular emphasis on ways to view and compare multiple maize genomes, billions of SNPs, and new ways to search and browse phenotypes, gel images, QTLs, and other data types. A key component to the redesign will be community involvement. To insure that the new interface is useful, guidance and beta testing groups will be created and consulted. Here we report our anticipated timelines and focus on initial concepts and designs, new features to be deployed (data, tools, and resources), and outline how you can be involved in changing MaizeGDB to better meet your needs.

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T17

Maize HapMapV2 - Capturing variation in a genome in flux

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The tremendous genetic diversity of maize manifests as high levels of nucleotide diversity not only in terms of single-nucleotide polymorphisms and small insertion-deletions, but also from copy-number and structural variation. For example, it has previously been reported that up to 30% of any maize inbred line is not orthologous to another, and a recent survey in maize and teosinte identified approximately 3800 segregating gene-level copy-number variations. In this extension of the maize hapmap project, we scale out the breadth and depth of the study by re-sequencing over a hundred inbred lines across the Zea mays, including the wild subspecies parviglumis and mexicana, and landrace and improved varieties of subspecies mays.. The sequences provided an average depth of 5X per inbred line and from these variant features were mapped to the reference genome. Given the complexity of the maize genome, we used two parallel but complementary strategies for mapping reads and identifying variation. These variations were validated using linkage-disequilibrium against an anchor set of high-confidence markers, as well as allelic agreement in regions that were identical-by-descent. In total over 50 million SNPs and indels were identified. Furthermore, by comparing the distribution of read depth across fixed genomic windows and genic regions, we identified polymorphic copy-number features. Finally, we demonstrated the utility of these SNP, indels and copy-number polymorphisms in genome-wide association mapping for quantitative traits in the NAM population. Results from this data will not only be valuable for high-resolution QTL mapping, but also highlight the significance of structural variation on phenotype variation.

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T18

Toward Fusarium ear rot resistant fumonisins-free transgenic maize for sub-Saharan Africa

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Fungal pathogen *Fusarium verticillioides* causes Fusarium ear rot of maize and also produces mycotoxins called fumonisins, which pose great health risks to humans and animals. Mycotoxin contamination of maize has been reported from at least 16 sub-Saharan African countries. Classified as group 2B carcinogens, their presence in maize-derived foods and feeds must be eliminated before consumption. Deployment of Fusarium ear rot resistant transgenic maize will provide small-holder farmers of Africa with economical control of Fusarium ear rot and fumonisins. We have identified two plant defensins, MsDef1 and MtDef4, and a virally encoded antifungal protein KP4 from the P4 strain of *Ustilago maydis*, that exhibit potent *in vitro* antifungal activity against multiple field isolates of *F. verticillioides*. These three antifungal proteins inhibit fungal growth using different modes of action. Several transgenic maize lines overexpressing each of these proteins separately were generated using a strong constitutive maize ubiquitin (*Ubi1*) promoter. Using conventional breeding we have also combined these three genes in all possible combinations. In the controlled field trials in North Carolina, a couple of 2-gene stacked lines (*MsDef1xMtDef4* and *MtDef4xKP4*) have shown significant improvement in Fusarium ear rot symptoms and fumonisins contamination. Based on growth chamber trial an additional 2-gene stacked line (*MsDef1xKP4*) has shown significant resistance to Fusarium ear rot.

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T19

Comprehensive association, linkage and mutant analysis implicate Tasselseed2 in defense response in maize

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Quantitative disease resistance is generally more durable than qualitative disease resistance, but much less is known about the mechanisms involved. Northern leaf blight (NLB) is a major fungal pathogen of maize of worldwide importance for which resistance controlled by loci with both qualitative and quantitative effects. As part of an effort to dissect the genetic and mechanistic basis for quantitative resistance to NLB, a SNP in the *Tasselseed2* (*Ts2*) gene was identified through association mapping in a ~300 maize inbred line diversity panel (Flint-Garcia *et al*., 2005). Re-sequencing of the *Ts2* gene in the diversity panel led to the identification of a significant non-synonymous SNP in linkage disequilibrium with the originally identified SNP. We confirmed the presence of a QTL for resistance to NLB in the *Ts2* gene region using selection mapping for allele frequency changes in an NLB recurrent selection population, and linkage mapping from a segregating population derived from the NLB recurrent selection population. A segregating *ts2* mutant population derived from 106E (*ts2-2409*), and a replicated experiment across two environments with the *ts2-ref* mutant in a W22 background were examined for resistance to NLB. In both experiments, the *ts2* mutants had a significant increase in NLB severity compared to their wild-type counterparts with a difference of 8% and 9% difference in average diseased lesion area for the *ts2-2409* and the *ts2-ref* experiments, respectively. TASSELSEED2 is an alcohol dehydrogenase in the jasmonic acid pathway determining sex differentiation in the floral architecture of maize. While the jasmonic acid pathway is recognized in plant defense, this is the first report implicating *Ts2* in disease resistance.

T20

Integrating MAGIC and NAM to enrich the genetic network underlying the maize immune response

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This project seeks to expand and enrich the genetic architecture of the HR (hypersensitive response), the plant kingdom's most important immune response. However, instead of using artificially induced variation to do so, we are making use of the variation that is present naturally in the maize germplasm. Our rationale for this derives from the fact that, despite the use of exhaustive mutagenesis screens in many plant species, our understanding of how HR is triggered and executed remains incomplete. One way forward is to exploit natural variation, which has been generated and selected over millions of years of evolution. However, a major challenge to this approach is how to sift through the enormous diversity available. To this end, we have devised a simple yet effective method to discover and characterize useful alleles. This method, a variation on enhancer/suppressor screening that we have called MAGIC (for Mutant-Assisted Gene Identification and Characterization), makes use of the phenotype of a mutant (for a gene affecting the trait of interest) as a reporter to discover and analyze relevant, interacting genes present naturally in diverse germplasm. Using a constitutively-active (semi-dominant) allele of the of the Rp1 disease resistance gene in a MAGIC screen of the diversity panel, an amazing amount of variation capable of enhancing or suppressing the HR response was revealed in the maize germplasm. Since B73 had a moderately suppressing effect on HR and Mo17 enhancing, it allowed us to conduct a MAGIC screen on the IBM RILs to uncover a major QTL. We have named it Hrml1 (HR modulating locus-1). Now we are integrating MAGIC with NAM not only to uncover additional Hrml loci but also to clone the genes underlying them. The identity of the genes/QTL thus identified will be confirmed by a combination of targeted EMS mutagenesis and/or transposon tagging techniques.

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T21

Genome-wide patterns of genetic variation among elite maize inbreds

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Whole genome resequencing approach has been successfully used in rice studies to facilitate molecular breeding. Genes related to growth, maturity, productivity or resistance have been identified and can be further applied in breeding programs.

With the advanced high-throughput sequencing technology and the availability of the reference maize genome, it has become feasible to resequence the entire genome of maize and thereby allow the genome-wide survey of genetic variation. In this study, we reported resequencing of six elite maize inbred lines, including the parents of the most productive commercial hybrid in China. More than 1.2 M SNPs have been found and this collection can be used to develop genetic markers to enhance breeding efforts and basic genetic studies. Over thirty thousand indel polymorphisms (IDPs) and 101 low-sequence-diversity chromosomal intervals have been uncovered in the maize genome. High-density SNPs and IDPs markers reported in this study are expected to be a valuable resource to explain the molecular basis of heterosis, and to identify QTLs for molecular breeding applications. Several hundred complete genes that exhibit presence/absence variation (PAVs) have also been identified among these resequenced lines. The complementation of PAVs and other deleterious mutations may be related with heterosis. Whole genome resequencing will have far reaching implications for improving breeding strategies and plant varieties to meet the world's growing demand on plant production and development.

T22

Ear Shoot Meristem Transcript Abundance and Grain Yield

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A whole-genome deep-sequencing transcript abundance scan for genotypic variation in gene expression in maize (Zea mays L.) ear shoot apical meristem was conducted on tissue sampled from a generation means experiment administered under field conditions. Additive variation was the most prevalent mode of genotypic variability observed, implying that regulation of gene expression was primarily cis-oriented. Highly significant genotypic variation in transcript expression was detected in over 25% of the 30,208 genes scanned. Of these, 3% were highly correlated with genotypic variation of kernels per row and grain weight per ear. Genes at the ramosa1 and ramosa2 loci, and a ramosa3 paralogue on chromosome 2, were conspicuous members of this set, substantiating that genotypic variation for lateral branch initiation in the ear shoot apical meristem is associated with variation of branching phenotype in the mature ear. A possible role of reactive oxygen signaling in conjunction with a CCAAT/CREB molecular switch controlling lateral branch initiation will be discussed.

T23

A systems approach to elucidating transcriptional networks that control maize inflorescence architecture

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Branching patterns of grass inflorescences are determined by developmental fate of highly organized stem cell populations called axillary meristems. Genetic control of branching in maize, especially in ears where kernels are borne, has clear relevance to grain yield and harvesting ability. In this work, we've taken a systems approach, combining genetics and genomics methods, to investigate transcriptional networks contributing to axillary meristem indeterminacy in maize. We used a high-throughput RNA-seq strategy to generate genome-wide transcript profiles that capture spatio-temporal expression changes during development in ear and tassel primordia, as well as changes resulting from genetic perturbation. The latter includes loss-of-function mutations in three key regulators of a developmental pathway controlling branching, the *RAMOSA* (*RA*) pathway. Functional *RA1* and *RA2* genes, which encode transcription factors, and *RA3*, a sugar metabolic enzyme, are essential for repressing branches. Expression signatures from developmentally staged ears of branching mutants were compared with those of tassel and sorghum inflorescences, all of which show distinct patterns and degrees of branching. We used the dynamic transcript profiles to identify genes co-expressed with *RA1* and *RA3* and enriched for specific functional classes or pathways. Approximately 50% of differentially expressed genes in *ra3* mutants were also altered in *ra1*. Co-expressed genes were also interrogated for upstream cis-elements and putative regulatory modules. We identified classes of transcription factors as putative candidates in the regulation of branching and that intersect multiple hormone pathways. In addition, small RNA profiles from ear and tassel were compared with those from *ra1* mutants to identify differential regulation of putative target genes in the RNA-seq datasets. We are further investigating genome-wide DNA occupancy profiles for *RA1* in relation to expression changes and regulatory elements. Collectively, these data provide a framework for elucidating key regulatory components of a developmental program with important implications for crop improvement.

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T24

A *narrow-leaf warty* phenotype and cell division anomalies result from mutations in maize *Cellulose Synthase-like D1*

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The *Cellulose Synthase-Like D* (*CslD*) genes have important, though still poorly defined roles in cell wall formation. They are hypothesized to encode cellulose or hemicellulose backbone synthases, though their specific biochemical activity remains unclear. Here we provide evidence for an unexpected involvement of maize *CslD1* in cell division. Pleiotropic effects of *csld1* mutants included narrow, rough-textured leaves, with "warty" lesions of ballooning epidermal cells, some expanding to a volume 75-fold greater than normal. Growth and dry weight of mutant plants were almost 45% less than non-mutants. Although mutant leaves were 35% narrower, epidermal pavement cells were wider, even in non-warty areas, reflecting 45% fewer cells across a leaf blade. Differences in cell-wall composition were not detectable for leaf blades or epidermal peels of *csld1* mutants, but high-resolution X-ray micro CT of mature stems showed thinner, though denser cell walls. Morphological defects of mutant plants were traced to a narrow temporal and spatial window in early leaf development, coinciding with highly-localized expression of the *CslD1* gene in zones of active cell division at the bases of growing leaf blades. In this region, evidence of atypical epidermal cell division included incomplete or erratically positioned cross-walls, as well as disruptions in cell file organization, cell shapes and sizes, and nuclear volume. Collectively, these data indicate a previously unrecognized role for CSLD activity in plant cell division.

Funding acknowledgement: National Science Foundation (NSF)

T25

The grass specific *liguleless narrow* gene and its paralog regulate leaf development in maize

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Maize leaves arise from the shoot apical meristem in a distichous pattern and differentiate into distinct tissue types along the proximodistal axis; the distal blade, proximal sheath, and the blade-sheath boundary where ligule and auricle exist. The semi-dominant mutant *Liguleless narrow* (*Lgn*) was isolated as a half plant chimera in a B73 EMS mutagenized population. *Lgn-R* heterozygotes have narrower and shorter leaves with a defective ligular region. Auricles are missing and ligules occur over the midrib but fail to extend to the margins. This defect in ligule and auricle initiates early in leaf development when the blade-sheath boundary is established, as a continous preligular band is not observed in *Lgn-R* heterozygotes. *Lgn-R* heterozygotes also display defects in reproductive development, such as reduced tassel branches, and failure in ear formation. These defects are much more severe as a homozygote. In order to understand the function of LGN in ligular region development, double mutants were generated with *lg1-R*. Results show that *Lgn-R* and *lg1-R* function synergistically to control ligule and auricle development. Positional cloning identified a grass specific Ser/Thr receptor-like kinase to be encoded by the *lgn* gene. A highly similar paralog is found in maize and other grasses. The *Lgn-R* mutation results in decreased kinase activity and increased transcript levels of the paralog.

Funding acknowledgement: CSREES

T26

The maize mutant *Tunicate1* is caused by ectopic expression of a MADS-box transcription factor, *Zmm19* gene in a dosage-dependent manner

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Pod corn was once regarded as the ancestral form of cultivated corn due to its characteristic of enclosing the kernel in glumes or chaff and was prized by pre-columbian peoples for its magical properties. The *Tunicate1* (*Tu1*) mutant of maize is a naturally-occurring dominant mutation, resulting in a striking pod corn phenotype that may be present in the ancestors of maize. In a dosage-dependent manner, we could observe not only the elongation of outer glumes in the tassel and the ear, but also the failure of selective abortion within the tassel and the formation of branch meristems within the ear. Based on sequence data of *zmm19* gene from *Tu1* mutants (presented by T. Muenster et al.), we confirmed that *Tu1* has the duplication of a maize MADS-box *Zmm19* gene whose 5'UTR region is invaded by unknown *mutator-like* DNA transposon, causing upregulation of Zmm19 gene. Here we show that YFP and RFP-tagged *Tu1* transgenic maize plants phenocopy *Tu1*. Also, YFP and RFP-fused Tu1 proteins are localized to the nucleus in mature vegetative tissues and immature reproductive organs, suggesting Tu1 protein participates in specifying a floral organ identity. Moreover, misexpression of miR172 is detected in *Tu1*, suggesting that *Zmm19* transcription factor may target *miR172* gene(s) in a direct or indirect way, a conclusion that is supported by the phenotype of miR172 transgenic lines. Interestingly, the 22Mb region surrounding the *tu1* locus in inbred B73 was selected for finishing during the maize genome sequencing project, and we have found that insertion of the *mutator-like* element resulted in a 1.8Mb inversion immediately upstream of *zmm19* gene in *Tu1*. This rearrangement prohibits recombination, and so likely accompanied the origin of this ancient gene.

T27

RAMOSA1 interacts with KNOTTED1 and regulates meristem determinacy via gibberellins during maize inflorescence development

(presented by Xiang Yang <yangx@iastate.edu>)
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RAMOSA 1 (RA1) is a plant-specific EPF-like protein with a Cys2-His2 zinc finger DNA binding domain and two EAR repression motifs that regulates branch architecture of the maize inflorescence. In *ra1-R* and *ra1-RSenh* strong mutants, both the tassel and the ear become highly branched due to loss of meristem determinacy. *ra1* RNA is expressed at the junction between those meristems and the main inflorescence axis, and we detected similar RA1 protein localization in transgenic maize expressing YFP-RA1 under the control of native promoter sequence. To elucidate the mechanism of RA1 action, yeast two-hybrid analysis was used to screen for RA1-interacting proteins encoded in young ear cDNA libraries. Several transcription factors including KNOTTED1 (KN1) were identified. The interaction between RA1 and KN1 was confirmed by GST pull down and bimolecular fluorescence complementation (BiFC) experiments, and mapped onto the domain structure of the two proteins. In tests for a genetic interaction, the tassel phenotypes of the *kn1-e1*; *ra1-RSenh* double mutants were statistically analyzed, which also supported an interaction between RA1 and KN1 in regulating inflorescence branch architecture. KNOX proteins are known to regulate gibberellin levels in lateral organ initiation, and *ga2 oxidase1* is a direct target of KN1. We found altered transcript levels of gibberellin biosynthesis genes in developing *ra1-R* mutant inflorescences, and that exogenous gibberellic acid 3 (GA3) treatment partially corrected the *ra1-R* mutant phenotype. Together, these results suggest a novel role for gibberellins in regulating axillary meristem determinacy, in the inflorescence.

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T28

Natural variation and new pathways in maize meristem size regulation

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Meristems, regulate their size by balancing stem cell proliferation with the incorporation of daughter cells into primordia. Analysis of the thick-tassel dwarf1 and fasciated ear2 (fea2) mutants in maize has shown that the CLAVATA signaling pathway is conserved in maize. Interestingly, fea2 maps to a QTL interval for seed row number, and to ask if fea2 is responsible for the QTL, we screened for hypomorphic, EMS alleles, via TILLING. We isolated four new alleles of fea2, all predicted to be weak. Analysis of seed row number in two of them showed a significant increase, proving that allelic variation at the fea2 locus can lead to changes in seed row number.
To characterize other factors in the meristem size pathway, we characterized compact plant2 (ct2), a classical mutant of maize. ct2 mutants are semi-dwarf with severe meristem-size defects, especially during inflorescence development. Using positional cloning, we identified ct2 as the alpha subunit of a heterotrimeric GTPase, which is involved in various hormonal signaling pathways in Arabidopsis and rice. CT2 expression was analyzed using transgenic lines expressing a CT2-YFP translational fusion under control of its endogenous promoter. We found CT2 to be present in the plasma membrane, and in tissues most affected in the mutant. We also present a potential link between CT2 and gibberellin signaling, based on the findings that the expression of a major GA-biosynthesis enzyme is decreased in the vegetative apex of ct2 mutants, and that endogenous GA levels are reduced. Furthermore, exogenous application of GA rescues the enlarged meristem phenotype of the mutant, indicating a novel connection between GA signaling and meristem size regulation. Our results suggest opposing modes of action for GA in internode elongation and in meristem size homeostasis.

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

T29

The roles of ZmMYB31 and ZmMYB42 in the regulation of the maize lignin biosynthetic pathway

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Two R2R3-MYB type transcription factors (TFs) *Zm*MYB31 and *Zm*MYB42 have been linked to the negative regulation of the lignin biosynthetic pathway by overexpression in *Arabidopsis*. These TFs exhibit 92% identity but their regulatory role and the extent to which they have overlapping function had not been determined in maize. Using SELEX assays, we defined the consensus DNA-binding site of *Zm*MYB31 and showed that it corresponds to the canonical AC-II element (ACC $^{T}/_{A}$ ACC) 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 *Zm*MYB42 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 *ZmA*1 genes promoters *in vivo*. However *Zm*Myb31 requires both ACII elements for efficient repression of COMT, while *Zm*MYB42 only needs the second upstream ACII element. Further results show that both proteins also bind to different targets, *Zm*MYB31 interacts with the *ZmF5H* gene promoter while *Zm*MYB42 interacts with the maize *Zm4CL2* gene promoter *in vivo*. The combined information arising from the characterization of *Zm*MYB42 and *Zm*MYB31 shows that these two factors play non-redundant functions in the regulation of the phenylpropanoid pathway, even though they are phylogenetically closely related. Moreover, these studies highlight the complexity of the phenylpropanoid pathway regulation and can inform strategies to modify lignin content in biofuel grasses. This project was funded by grant NSF DBI-0701405 and the Ohio Plant Biotechnology Consortium (OPBC).

Funding acknowledgement: National Science Foundation (NSF), Ohio Plant Biotechnology Consortium (OPBC)

T30

Engineering N Storage Capacity of Plants to Improve NUE

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The global demand for nitrogen (N) fertilizer for agricultural production, which already stands at ~90 million metric tons per year, is projected to increase to 240 million metric tons by the year 2050. A significant portion of applied soil N is lost by leaching, run-off and de-nitrification, which not only adds to the cost of crop production but also to the pollution of the environment. Developing crop varieties that are more efficient in absorbing and utilizing N will help mitigate these problems. We have been exploring various approaches in our efforts to improve N use efficiency of maize and other crop plants. One of these is the addition of a transient N storage mechanism into the plant that would increase its N storage capacity, which could be useful for plant growth during periods of N deficiency. By subjecting maize seedlings to high N in the growth medium, we identified a lipoxygenase (Lox6) protein that exhibited the characteristics of a vegetative storage protein. Lox6 was localized to chloroplasts of the mesophyll cells even though it does not possess a discernible organeller targeting signal. Upon over-expression of *Lox6* under the control of various promoters and targeting signals, an order of magnitude more Lox6 protein accumulated in the leaf tissue without any detectable, detrimental effect on the other, major leaf proteins. Whether it was expressed in the mesophyll or bundle sheath cells, the transgenic protein accumulated in the chloroplasts. Lox6 remobilized from the leaf like the other proteins during senescence.

T31

Diverse roles of jasmonic acid in reproductive development and defense as revealed by the analyses of the JA-deficient OPR mutants

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Oxo-phytodienoate reductases (OPR) are enzymes involved in the biosynthesis of the hormone jasmonic acid. Null mutants of the *ZmOPR7* and *ZmOPR8* genes were generated using *Mutator* insertional mutagenesis. Functional analysis of the single and double mutants revealed both redundant and unique functions of these isoforms and diverse roles of JA in maize defense and development. *opr7opr8* double mutant (DM) has dramatically reduced levels of JA in the organs tested while only *opr7* was responsible for JA pulse in wounding response in leaves. The DM displayed feminized/ male-sterile *tasselseed* phenotype reminiscent of *tasselseed1* mutant phenotype and this feminization can be reversed by JA, providing genetic evidence that JA is an essential signal for male sex determination in maize. However, unlike *ts1* mutant which is not defense-compromised to pathogens and insects, *opr7opr8* DM is nonviable under field or non-sterile soil conditions due to extreme susceptibility to root rotting oomycete *Pythium* spp., indicating that JA plays a vital role against *Pythium* damping-off disease. Additionally, the DM displayed a number of other JA-related phenotypes: lack of anthocyanin pigmentation of brace roots, delayed leaf senescence and elongation of ear stalk. To identify JA-dependent genes in maize response to mechanical wounding, we used macroarrays spotted with gene-specific probes for 85 diverse JA related or defense genes including all 13 lipoxygenase (LOX), eight OPR, and 19 JAZ gene family members. The results showed that wound-inducible JA biosynthesis genes like *LOX8*, *AOS* and *AOC*, as well as JA signaling genes such as *JAZ7* and *JAZ8*, and defense genes like *LOX2, LOX3, HPL, PR5, MPI, WRKY46, PAL2* and *PAL3* are substantially down regulated in the DM mutant versus wild type in response to wounding. These data suggests that wound induction of all these genes is JA-dependent and relevant to defense and development of maize.

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T32

Maize threonine synthase mutants cause an embryo-lethal, rough endosperm phenotype

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Transposon-tagging has been used extensively as an approach to clone maize mutants. The completion of the B73 genome sequence has made map-based cloning practical in maize. However, map-based cloning is still labor-intensive and transposon-tagging is high risk since not all mutant alleles from transposon populations are tagged. We are integrating transposon-tagging with genetic mapping to increase the rate at which maize seed mutants can be analyzed. We are focusing on maize rough endosperm (rgh) mutants from the UniformMu transposon-tagging population. We generated a transposon flanking sequence tag (FST) resource that identified 5,000 non-redundant insertions from 144 rgh mutants. In parallel, we are developing mapping tools to rapidly map mutants from UniformMu by crossing to the B73 or Mo17 inbreds. Using a set of 85 distributed Simple Sequence Repeat (SSR) markers giving nearly complete coverage of the maize genome, we mapped a rgh mutant to the long arm of chromosome 6. This map position co-localized to an FST corresponding to the seed mutant isolate. An independent allelic insertion was found within the FST resource, and the alleles co-segregate with embryo lethal, rough endosperm phenotypes that fail to complement in crosses. The locus identified encodes chloroplast-localized threonine synthase (TS). TS converts O-phosphohomoserine (OPHS) into threonine (thr). OPHS is also synthesized indirectly into methionine (met) by cystathionine gamma-synthase (CgS), which competes directly with TS for a common precursor. Met is an essential amino acid in animal diets, including humans. Downregulation of TS expression has been a target to increase Met levels in dicot systems. The maize loss of TS phenotype suggests that similar strategies will not be effective unless TS is expressed in the embryo.

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T33

Genome-wide association study of carbon and nitrogen metabolism in the maize nested association mapping population

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Carbon and nitrogen metabolism is critical to plant growth and development. We evaluated the maize nested association mapping population for quantitative variation of 12 carbon and nitrogen metabolites. In total, about 12,000 samples and more than 100,000 assays were processed with the robotized platform. Using joint linkage analyses, common QTL were identified for different metabolites especially for those with related pathways. We found that the correlations between different metabolites are driven by the proportion of shared QTL. Therefore, we were able to build up the relationships of the studied metabolites based either on their correlations or proportion of shared QTL. Using genome wide associate study (GWAS), we identified SNPs from different candidate genes associated with various metabolites. For example, in one case, we identified SNPs from *carbonic anhydrase* genes associated with multiple different metabolites in nitrogen metabolism and photosynthesis pathways; in another case, SNPs from *invertase* genes associated with metabolites from starch synthesis pathway. We demonstrated here that joint linkage analysis combined with GWAS in maize NAM population can be an efficient way to dissect natural variation in conserved metabolic pathways.

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T34

Identification of regulatory elements of terpene biosyntheses by Nested Association Mapping (NAM) and Genome Wide Association Study (GWAS)

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Plant secondary metabolites can serve as plant defensive compounds or mediators of chemical communication, e.g. as attractants for natural enemies of herbivores. Maize plants attacked by caterpillars release a mixture of mono- and sesquiterpenes that attracts parasitic wasps, which are specific enemies of the herbivores. In maize roots, the attack of Western Corn Rootworm induces the emission of the sesquiterpene *(E)*-β-caryophyllene which attracts entomopathogenic nematodes. In our effort to study the molecular base of these indirect defense mechanisms, we want to identify the genes responsible for volatile terpene biosynthesis as well as their regulatory elements.

About 5000 recombinant inbred lines of a Nested Association Mapping (NAM) population derived from 26 inbred lines were screened for herbivore induced volatile production. The variation of volatile production within the NAM population enabled us to identify a set of important quantitative trait loci for volatile terpene production by nested association mapping (NAM). Genome wide association study (GWAS) utilizing a large SNP population resulted in close mapping of several QTLs. Some of the QTLs correlate with genes that encode enzymes responsible for the biosynthesis of a specific terpene, for example in the production of the sesquiterpenes nerolidol and 3,8-dimethyl-1,4,7-nonatriene. For *(E)*-β-caryophyllene production, at least one QTL is associated with regulatory elements that up-regulate the production of the volatile after herbivore damage. Comparison of the mapping results with the terpene metabolites of the parent lines enables us to characterize the pathways and their regulatory mechanisms.

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T35

Segregation of Non-allelic Homologs Generates Transgressive Variation in Genome Content

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A genome-wide analysis identified novel CNV within two maize recombinant inbred lines (RILs) derived from the inbred parents, B73 and Mo17. These examples of de novo copy number variation include both gains and losses in the RILs relative to their inbred parents. These gains and losses were validated via both PCR-based methods and whole exome array capture-and-sequencing experiments. In total, 185 genomic regions (which overlap with 38 high confidence genes from the filtered gene set (4a.53)) exhibit novel CNV in the two analyzed RILs. The largest CNV region is >50 kb. Evidence was found for a high rate of recurrent de novo CNV because 12 of these genomic regions exhibited novel CNV in both RILs. Further analyses demonstrated that the observed CNVs were in fact derived via meiotic segregation of mostly single copy homologous sequences that are located in non-allelic positions in the two parental haplotypes. Consequently, in the F1 these two loci were both hemizygous and expected to produce F2 lines (and RILs) containing zero, one or two copies. This finding provides an alternative explanation for the origin of de novo CNVs. In addition, significant associations were identified between phenotypic QTL and genomic loss or gain of at least two of 14 tested pairs of non-allelic homologs. Thus, this mechanism may contribute to transgressive segregation typically observed in the progeny of bi-parental crosses.

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Poster Abstracts

P1

Gramene: A Resource For Comparative Plant Genomics

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The Gramene database (http://www.gramene.org) is a curated data resource for comparative genome and functional analysis in over 10 plant species, including Zea mays. Through integration of genomic sequence, genes, proteins, genetic and physical maps, germplasm, genetic and phenotypic diversity, and biochemical pathways, we provide an unparalleled framework for carrying out maize research in the context of other crops. To index and combine data from multiple plant species, Gramene makes extensive use of ontologies (controlled vocabularies), including those for plant structures and growth stages, traits and phenotypes, gene function, biological processes, cellular components, and environments. All data in Gramene is publicly available, and all code is open source. Online tutorials and help documents provide users with an overview on how to conduct a wide variety of analyses on the database, and an interactive helpdesk supports individual queries.
Release 32 (Fall 2010) includes complete genome sequences of five monocots (rice subspecies *japonica* and *indica*, sorghum, *Brachypodium*, and maize), four dicots (*Arabidopsis*, *A. lyrata*, grape, and poplar), the moss *Physcomitrella*, and partial genomes of several wild rice species. New data includes annotations, FGenesH gene predictions, Compara gene trees, and genetic diversity data supplied by contributing rice, Arabidopsis, and maize databases. New features include multi-species views, synteny maps based on phylogenetically-determined orthologs, multiple whole-genome alignments, and ancestor reconstruction using the Enredo/Pecan/Ortheus pipeline. These data and features are fully integrated with other Gramene resources, including gene and protein-level annotations, GO ontology, genome browsers, diversity data, and pathways. We also provide links from our website to external analysis tools such as Tassel and Flapjack, and updated our genome browser to Ensembl version 60. Additionally, we added beta versions of the BrachyCyc and MaizeCyc metabolic pathways databases. Work is currently underway to generate an updated version of MaizeCyc based on the Maize RefGen v2 Filtered Gene Set (TBA by the Maize Sequence Project) and the latest phylogenetic Compara gene trees.

Gramene is supported by a grant from the NSF and represents a collaborative effort between Cold Spring Harbor Laboratory, the Department of Plant Breeding and Genetics at Cornell University, Ensembl Genomes, and various national and international projects dedicated to cereal genomics and genetics research.

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P2

In search of new genomic regions involved in maize domestication

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Maize (*Zea mays* ssp *mays*) was domesticated from teosinte (*Zea mays* ssp *parviglumis*) in Central Mexico close to 9,000 years before present. Although several important loci that contributed to the architectural changes that modified teosinte into maize have been identified, many of the genetic and environmental factors that shaped this transformation remain largely unknown. Public access to the maize genome sequence offers a possibility for conducting large-scale comparisons of genomic structure between improved inbred lines (B73 and Mo17) and Mexican landraces (Palomero). We have identified **N**early **I**dentical **S**equence **R**egions (NIdSR) between Palomero and B73 that show at least 95% identity and 100% homology in a length of no less than 200 base pairs (bp). For each unique NIdSR, we have determined its genomic location, frequency of recombination, gene content, and eventual genomic redundancy. As expected, the distribution of NIdSRs in B73 genomic segments of 150 Kb suggests a differential distribution, with large genomic regions showing total absence of NIdSRs, and regions corresponding to organellar genomic insertions showing high degree of sequence conservation. A specific group of genomic regions shows normal to high recombination frequencies associated to high content of NIdSRs, suggesting that at least some of them could represent regions affected by artificial selection. We are currently conducting a detailed analysis of nucleotide variability in some of these regions to determine a possible involvement in domestication. This work is being supported by grant ZEA-2006 (SAGARPA), and CONACyT.

Funding acknowledgement: SAGARPA, CONACyT

P3

A meta-analysis of QTL associated with ear rot resistance in maize

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Maize ear rot (ER) is one of the most prevalent types of maize disease worldwide. The three predominant ER diseases, Aspergillus ear rot (AER), Fusarium ear rot (FER) and Gibberella ear rot (GER), are responsible for the most disease-related reductions in yield and quality. To identify QTL hot chromosome regions for the three ER resistances and to investigate the relationship among these ER resistances, 87 initial QTL from 14 studies were projected on a high-density genetic linkage map (IBM2 neighbors 2008). Meta-analysis showed that 29 meta-QTL (MQTL) comprising two to six initial QTL were located on chromosomes 1 to 8. Of them, six most promising MQTL were located on chromosomes 3 and 4. They were recommended to use for improving ER resistance in a marker assisted selection program. Meta-analysis also indicated that some QTL from different ER types or genetic sources were clustered on the same chromosome regions, particularly on bins 3.04 and 2.08. Among eight resistance sources, CO387 contributed 18 of 29 MQTL.

P4

Automated Functional Annotation of Maize Genes Using a Support Vector Machine

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An ongoing series of investigations has identified a set of over 6,000 *Zea mays* genes of interest that play a role in shoot apical meristem (SAM) function and leaf primordia development. High-quality manual functional annotations for over 1,000 of these genes has resulted in a database of gene ontology (GO) numbers as well as EC numbers for genes that encode enzymes (http://www.genome.jp/kegg/). These annotations are being used as the training set for an automated functional annotation system using a support vector machine (SVM). The system uses cDNA sequences as BLASTx queries at GenBank/NCBI to identify relevant articles in PubMed; SVM input attributes are harvested from the abstracts of these articles in order to predict GO terms, primarily in the biological process sub-ontology. Current work is focused on improving the number and quality of attributes and on increasing the selectivity of abstracts associated with a given cDNA sequence.

Funding acknowledgement: National Science Foundation (NSF)

P5

De novo assembling of Mo17 genome using short reads from Solexa sequencing

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Maize is a species that exhibits exceptional genetic diversity among different individuals. Although the whole genome sequence information of B73 is now available, there still exists large practical need to have genome of some key representative inbred lines being completely sequenced. Mo17 is such a key inbred line. The genome sequence of B73 inbred line was accomplished using Sanger sequencing under BAC-by-BAC strategy. In our study, we report the whole genome sequencing of Mo17 inbred line using Solexa sequencing technology. A total of over 160 Gb short reads rang from 75 bp and 120 bp were generated using paired-end sequencing libraries of various fragments ranging from 200 bp, 500 bp, 2kb and 5kb.De novo assembling was done with parallel assembler which balances in speed and computing resource usage. Details of the assembling results for Mo17 inbred line as well as its genome-wide comparison with B73 inbred will be presented during the meeting.

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P6

Development of a computational pipeline to identify and classify non coding RNAs in maize

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Transcriptome analysis has revealed a significantly larger amount of non-coding transcripts than protein-coding transcripts in the genomes of many species. Small non-coding RNA are well studied and have been grouped into various classes (i.e. siRNA, miRNA) based on length and function. Long non-coding RNAs (lncRNAs)are much more elusive, although their abundance and functional importance in mammals is becoming increasingly clear. While studies of lncRNA have been conducted in some plant species, very few lncRNAs have been identified in maize. We are using the programming language Python to develop a pipeline that will identify lncRNAs from transcribed sequences and sort them into various classes. 134,099 sequences from the working gene set of RefGen_v1, made available through the Maize Genome Sequencing Project, were read into the Python pipeline. After removing those with open reading frames greater than 110 aa, 29,975 sequences remained. Further removal of sequences with homology to known proteins left approximately 10,000 sequences. These sequences will be further sorted into classes that have been defined according to potential non-coding RNA functions. Criteria for classification have been designed to distinguish siRNA and miRNA precursors, anti-sense lncRNA, intronic lncRNA, intergenic lncRNA, and predicted polycomb complex binding domain-containing lncRNA . A subset of lncRNAs identified by this study will be further characterized to determine their function. Understanding non-coding transcript function will increase our understanding of the maize genome as a whole.

Funding acknowledgement: National Science Foundation (NSF)

P7

Evidence for megabase-scale inversion polymorphisms in wild and domesticated *Zea mays* based on SNP genotyping data

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We report an examination of regions in the *Zea mays* genome that harbor unusually high levels of linkage disequilibrium (LD). Five large regions with high LD were identified in SNP genotyping data in the domesticate *Zea mays* ssp. *mays*, its wild progenitor ssp. *parviglumis*, and the weedy taxon ssp. *mexicana*. Combined with evidence of high LD over extended regions, data on sequence diversity and divergence suggest that at least three of these regions are chromosomal inversions. The putative inversions on chromosome 1 and 8 are longer than 10 Mb, and comparison to outgroup taxa suggests both are derived inversions specific to *Zea mays*. Haplotype diversity and LD within local *Zea mays* populations suggest the putative inversions on chromosome 1 and 8 are both segregating in populations across the natural geographic range of the species.

Funding acknowledgement: National Science Foundation (NSF)

P8

Genomic effects of local adapation in *Zea mays* ssp. *parviglumis* populations

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Zea mays ssp. *parviglumis* (hereafter *parviglumis*) is the closest wild relative of maize, endemic to southwest Mexico. *Parviglumis* populations are often dense and geographically distinct, growing in mesic middle-elevations (400-1700 m). These populations vary greatly in a number of environmental and ecological characteristics (exposure, slope, soil type, competition) and show considerable morphological and phenological variation. Therefore, populations likely show signatures of local adaptation.

Our aim is to identify genomic regions affected by local adaptation and characterize the traits and genes involved. We genotyped 12 individuals each from 10 *parviglumis* populations for more than 55 000 SNPs using the Illumina Infinium HD genotyping platform. Populations were chosen to span the geographic distribution and genetic diversity of the species. We will use F_{ST} -outlier methods to detect genomic regions that are exceptionally diverged among populations due to local adaptation or other population-specific selective events.

Parviglumis is an ideal organism to study the effects of natural selection on genomic variation. It is a wild, outcrossing plant in which selection is expected to be efficient due to high effective population size. In addition, the maize genome sequence and various genomic tools developed for maize are applicable to *parviglumis* and represent an enormous resource for disentangling the complexities of local adaptation.

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

P9

Improving the B73 reference genome via genotyping by sequencing (GBS)

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Next generation sequencing provides a new, cost-effective means to genotype large numbers of individuals of virtually any organism at high density. Genotyping by sequencing (GBS) can be achieved by targeting a reduced fraction of the genome such as sequence adjacent to restriction enzyme cut sites. We have demonstrated GBS in maize by genotyping 275 RILs from the IBM mapping population, filtering for sequence adjacent to *Ape*KI sites. We genetically mapped individual, 64-base GBS tags as presence/absence markers versus a new, high density map of 239 IBM lines produced from data from the Illumina MaizeSNP50 Genotyping BeadChip. We uncovered 485,860 GBS tags that could be genetically mapped at a binomial p-value <0.001. Using a high stringency ($p<10^{-7}$) subset of these, we found that, of the tags that segregated with B73, only 0.4% genetically mapped to a chromosome different from the one to which they physically align on B73 RefGen_v2. In contrast, for tags that segregated with Mo17, the comparable proportion was 9.3%. This indicates that the physical position of homologous sequences that segregate in a Mendelian fashion can differ greatly among lines: the maize genome appears to be in constant flux. We have also used the segregation data from the above 485,860 GBS tags to genetically map (1) contigs from chromosome 0 of B73 RefGen_v2, (2) contigs from *de novo* 454 sequencing of B73, and (3) full length cDNAs. Because the GBS data coverage for the IBM population was low (~0.4x), segregation data from multiple GBS tags per contig (or FLcDNA) were merged where possible to increase power. We were thus able to genetically map 8 of the 17 chromosome 0 contigs, 3408 novel 454 contigs and 407 FLcDNAs that are not represented in B73 RefGen_v2. Segments of the B73 reference genome to which elevated proportions of novel 454 contigs genetically map are prime targets for future improvement.

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

P10

Using a Support Vector Machine to Predict Nucleosome Occupancy Likelihood (NOL) in the Maize Genome

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Understanding the functional organization of the genome remains one of the biggest challenges in biology. Eukaryotic DNA is packaged in chromatin whose fundamental subunit is the nucleosome: ~150bp of DNA wrapped around a histone octamer. The position and density of nucleosomes plays a key regulatory role and is controlled by both chromatin regulatory complexes and by features intrinsic to DNA sequence. Recent work has described nucleosome occupancy in animals, yet this information is essentially nonexistent in plants. We hypothesized that information from human-based computational models of nucleosome occupancy may be used to identify regulatory elements in the recently sequenced maize genome. Nucleosome mapping predictions in maize were made using support vector machine (SVM) software that was trained on human chromatin. Nucleosomal occupancy likelihood (NOL) plots were able to identify canonical chromatin structural features at multiple scales of resolution. Viewed at the single gene scale, the NOL plots reveal typical transcription start site (TSS) "peak-trough-peak" signatures, as well as recently described signals at exon boundaries. At the 100Mbp scale, NOL plots are ideal for genome annotation and visual scanning, as well as pinpointing the location of genes and mobile repetitive elements. Viewed at the whole chromosome scale, NOL plots reveal expansive features that correlate with gene or retrotransposon density. Thus, we present a proof-of-principle for the annotation of a newly sequenced genome. Continuing this work will involve the training of a new SVM using nucleosome occupancy data from the Maize genome, allowing for comparative studies between it and the Human trained model. This research is expected to lead to useful annotation of the maize genome, uncover fundamental attributes of plant chromatin structure, create testable models of nuclear architecture, and establish a new paradigm for understanding the structure of the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

P11

Maize global transcriptomics reveals pervasive leaf diurnal rhythms but rhythms in developing ears are largely limited to the core oscillator

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Diurnal rhythms are plant vital environmental adaptations to coordinate their internal physiological responses to alternating day-night cycles. A comprehensive view of circadian biology has been lacking for maize (Zea mays), a major world crop. Genome-wide transcript profiling of maize leaves and developing ears was conducted on a high-density 105K Agilent microarray to investigate diurnal rhythms. In both leaves and ears the core oscillators were intact and diurnally cycling. Maize core oscillator genes are found to be largely conserved with their Arabidopsis counterparts. In leaves there is diurnal gene regulation, with some 23% of expressed transcripts exhibiting diurnal behavior. These transcripts can be assigned to over 1700 gene ontology functional terms, underscoring the pervasive impact of diurnal rhythms on plant biology. Considering the time at which each diurnally regulated gene expression peaks, and their corresponding functional assignments, most gene functions display temporal enrichment in the day, often with distinct patterns, such as dawn or mid-day preferred, indicating that there is a staged procession of biological events undulating with the diurnal cycle. Notably, approximately a fifth of gene functions display a bimodal enrichment flanking the mid-day photosynthetic maximum, with an initial peak in the mid-morning and followed by another peak during the afternoon/evening. In contrast to leaves, in developing ears as few as 47 gene transcripts are diurnally regulated and this set of transcripts includes primarily the core oscillators. In developing ears, which are largely shielded from light, the core oscillator therefore spins in isolation with little outward effect. The implications of these findings to physiological processes and source-sink tissue relations are discussed.

P12

The Maize Genome Project: Past, Present, and Future

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Primary sequencing of the maize B73 genome was completed in 2008, but work on the genome continues. In 2010 the sequencing consortium released a second version of maize genome reference assembly (RefGen v2). We also released an annotation build that improves upon the first set, including a working set of 110,028 genes and a filtered set of 45,348 genes, all available on MaizeSequence.org (Release 5b). We are also in the midst of a large research undertaking, in collaboration with the Buckler lab, to capture gene space missing from the reference through a *de novo* assembly of a whole-genome shotgun 454 library furnished by the Genome Center at Washington University. We've built gene scaffolds on over 8,000 full-length cDNAs previously unmapped in the reference, and are able to anchor a majority of them on the genetic map. We are also working closely with Gramene and with MaizeGDB to ensure continuity of the project and the online resources for maize research. This work was funded by the NSF/DOE/USDA "Sequencing The Maize Genome" project (NSF #0527192).

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

P13

Rapid genotyping of soybean cultivars using high throughput sequencing

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Soybean breeding involves constantly improving commercially grown varieties by introgressing important agronomic traits from poor yielding accessions and/or wild relatives of soybean while minimizing the associated yield drag. Molecular makers associated with these traits are instrumental in increasing the efficiency of producing such crosses. Single Nucleotide Polymorphisms (SNPs) are particularly well suited for this task as they are present at a high density in the non-genic regions of the genome thus increasing the likelihood of finding a tightly linked marker. Therefore a rapid method to develop SNP markers that can differentiate specific loci between any two parents is highly desirable. In this study we investigate such a protocol for developing SNP markers between multiple soybean accessions and the Williams 82 genome sequenced by DOE-JGI. To restrict sampling frequency, thus increasing depth, reduced representation libraries (RRLs) of genomic DNA were generated by restriction digestion followed by library construction. We chose to sequence four accessions Dowling (PI 548663), Dwight (PI 597386), Komata (PI200492) and PI594538A for their agronomic importance as well as Williams82 as a control. MseI was chosen to digest genomic DNA based on its propensity to cut less often in the high copy number regions of the genome, as defined by an earlier study. All RRLs were submitted for sequencing on the Illumina genome analyzer. Reads were aligned to the Glyma1 reference assembly and SNP calls made from the alignments. We identified from 4294 to 14550 SNPs between the four accessions and the Williams reference. In addition a small number of SNPs (1142) were found by aligning Williams 82 reads to the reference assembly (Glyma1) suggesting limited genetic variation within this line. The SNP data allowed us to estimate genetic diversity in the soybean germplasm. In addition the genomic data obtained in the process allowed estimation of differences in repeat composition between the lines. Restriction digestion of soybean genomic DNA with MseI followed by high throughput sequencing provides a rapid and reproducible method for generating SNP markers between accessions. We also discovered larger than expected amounts of residual heterogeneity widely distributed in the Willams82 accession.

Funding acknowledgement: United Soybean Board

P14

RNA-seq Reveals Novel Aspects of the Maize Gametophytic Transcriptomes

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Both male and female gametophytes play central roles in sexual plant reproduction. Furthermore, a hallmark of the plant life cycle is that gene expression is required in the haploid gametophytes, as many mutant phenotypes are expressed in this phase, affecting transmission of the mutant allele. However, the relative inaccessibility of the female gametophyte has limited the availability of genome-scale data on this structure. We have taken advantage of the relatively large size of the maize female gametophyte to sequence replicated RNA-seq libraries from dissected B73 embryo sacs, comparator ovules, mature pollen, and seedlings (as a baseline), using the Illumina platform. An initial analysis of these data has identified a set of empirically-predicted transcript-producing loci that show significant enrichment in embryo sac, mature pollen, or seedling samples. In this initial dataset, the embryo sac-enriched loci are 3-fold more likely than the seedling-enriched loci to map to portions of the genome that are not associated with transcript models from the 4a.53 Working Set; pollen-enriched loci are 9-fold more likely. Thus, the potential for gene discovery appears high in these samples. However, 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. Further analysis of these RNA-seq data assembled relative to the B73_v2 genome, as well as comparison to the new 5a.59 gene set, will be presented.

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P15

Pan-grass synteny: reciprocal gene deletion did not drive the radiation of the major grass species

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The grasses, Poaceae, are one of the largest and most successful angiosperm families. Like many species radiations, the divergence of the major grass lineages was preceded by a whole genome duplication (WGD) 50-70 million years ago. Using four sequenced grass genomes (maize, sorghum, rice and bracypodium), we generate a pan-grass syntenic dataset, and separated the two paleo-subgenomes derived from the pre-grass WGD. These data provide a complete list of all syntenic gene sets among these genomes, and also provide the opportunity to track the post-WGD evolution of genome content and their impact on speciation. Reciprocal loss of duplicated genes or genomic regions has been hypothesized to facilitate speciation. Of more than 6,000 pan-grass syntenic gene sets identified among these genomes, less than 0.6% show deletion of reciprocal homeologous gene copies in different species. Analysis of a subsequent WGD in the maize lineage (5-12 million years ago) that also post-dates the divergence with the sorghum lineage (12 million years ago), reveals no evidence of large scale deletions following WGD. These results strongly suggest that reproductive barriers created by gene loss following whole genome duplication did not drive the radiation of grass species. Instead, the correlation of plant radiations following WGD may instead be explained by evolution innovations enabled by the co-option of duplicated genes and networks into new functional roles. The pan-grass syntenic dataset is available for download at http://genomevolution.org/wiki/index.php/Syntenic_gene_sets, and maize genes in syntenic sets are highlight in CoGe's maize (v2) genome browser http://genomevolution.org/CoGe/GenomeView.pl?z=6&x=650000&dsgid=9106&chr=1 .

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P16

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 (*Zm*THX1) was cloned as a His-tag fusion protein in pDEST17 for study of it's preferred binding specificity. This project was funded by grant NSF DBI-0701405.

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P17

Plant Genomic Resources at National Center for Biotechnology Information

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Plant genomics is a simple expansion of the scope of genomics at the National Center for Biotechnology Information (NCBI). In addition to the tools for storage of and analysis of INSDC nucleotide sequence such as, respectively, GenBank and BLAST, genomics at NCBI includes databases that enable 1) monitoring the progress of genome sequencing projects (Entrez Genome Projects), 2) datamining of probes (Entrez Probes, UniSTS), 3) datamining of gene information (Entrez Gene), 4) defining gene-specific sets of cDNA by clustering (UniGene), 5) viewing genome units and the underlying components (MapViewer, CloneDB/CloneFinder) and 6) databases that allow datamining of NCBI-generated data (RefSeq and Plant Protein Clusters). These standalone tools are enhanced at NCBI by the capability to move among these and other databases as the data associations dictate. The pan-organism resources are supplemented by plant-specific resources: plant text search, PlantBLAST, and plant-EST BLAST. PlantBLAST provides organism-specific databases composed solely of the accessions associated with mapped loci visible through MapViewer. EST-BLAST provides plant-specific databases composed solely of the ESTs from those plants with more than 40,000 ESTs.

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P18

Sequence, Structure and Dynamics Analysis of Thermostability in Endoglucanases

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Endoglucanases are crucial enzymes used in the production of biofuels from cellulosic biomass, a process which requires thermostability at high processing temperatures. Despite the economic importance of these industrial proteins, we currently lack a basic understanding of how some endoglucanases can efficiently function at elevated processing temperatures, while others with the same fold have substantial reduction in activity. Here we explore the origins of thermostability in endoglucanases from sequence, structure, and dynamics perspectives using thermostable and mesostable protein sets. We performed a comparative sequence and structure analysis for thermophilic and mesophilic endoglucanases in (α/β)8, β-jelly roll, and (α/α)6 folds, followed by a dynamics analysis of the (α/β)8 fold using elastic network models. We observed that thermophilic endoglucanases and their mesophilic counterparts differ significantly in their amino acid compositions. Interestingly, these compositional differences are specific to protein folds and enzyme families and lead to modification in hydrophobic, aromatic, and ionic interactions in a fold-dependent fashion. We then focused specifically on a pair of thermostable and mesostable endoglucanases for a detailed dynamics analysis. It is often the case that thermophiles have shorter loops than their mesophilic counterparts, which was suggested to impart thermostability. In our case, however, the thermophile surprisingly possessed three insertions in the mesophilic loop regions and therefore has longer loops. The comparative structural dynamics analysis using elastic network models of (α/β)8 fold indicate that these three loops may contribute to the thermostability by modulating the direction of correlated motions between the catalytic residues (acid/base donor and nucleophile). We also observed that the thermostable protein showed larger dynamic domains than its mesostable counterpart, which suggests that cooperative dynamics is a critical contributing factor to thermostability.

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P19

Reinventing MaizeGDB

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The Maize Database (MaizeDB → MaizeGDB) turns 20 this year, and such a significant milestone must be celebrated! With the release of the B73 reference sequence and more sequenced genomes on the way, the maize community needs to address various opportunities and challenges. MaizeGDB, the community's Model Organism Database is in an excellent position to address evolving needs by deploying tools that will allow researchers to make use of multiple genome sequences as well as large-scale phenotypic associations. To prepare for both current and future needs of the community, the MaizeGDB team is planning a complete interface redesign to keep the resource relevant and to allow for expansion in desired directions while continuing to update the data as usual over time. The redesign will be both cosmetic and functional. The overall goal of the redesign is to create a clean modern interface with improved user interaction while expanding the overall functionality of MaizeGDB. Cosmetically, we will modernize the appearance and simplify page organization and navigation. Functionally, we will put particular emphasis on ways to view and compare multiple maize genomes, billions of SNPs, and new ways to search and browse phenotypes, gel images, QTLs, and other data types. A key component to the redesign will be community involvement. To insure that the new interface is useful, guidance and beta testing groups will be created and consulted. Here we report our anticipated timelines and focus on initial concepts and designs, new features to be deployed (data, tools, and resources), and outline how you can be involved in changing MaizeGDB to better meet your needs.

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P20

The MaizeGDB Genome Browser: Tools and Resources

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MaizeGDB (http://www.maizegdb.org) is the research community's database for maize genetics and genomics. B73 RefGen_v2, the latest assembly of the reference genome, has been available via the MaizeGDB Genome Browser since May of 2010 and is well-integrated with structural and functional annotations. New data types for v2 include Agilent and NimbleGen microarray tracks (in collaboration with the Walbot and Kaeppler Groups, respectively), a Gene Model Quality track (in collaboration with the Brendel PlantGDB Group), a leaf transcriptome track (in collaboration with the Brutnell Group), and HeritableMu insertions from the Barkan and van Wijk Mu-Illumina Group. Of particular interest to many is the tool we are developing called ZeAlign. It allows sequence-based matching of large datasets, e.g., SNP and RNAseq datasets, to the B73 reference genome assemblies. ZeAlign is intended to serve as a pipeline to get your sequence-based data aligned to the reference genome and ready for incorporation into MaizeGDB. To try out ZeAlign, visit http://zealign.maizegdb.org

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P21

POPcorn: A PrOject Portal for corn

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The rapidly increasing quantity of maize data available on the Internet means that locating and searching relevant data and information is increasingly difficult for maize researchers. Finding appropriate sites then learning how to navigate the sites can be difficult and time-consuming. Finding information associated with a particular sequence is likewise challenging, sometimes involving BLASTs at multiple sites and database searches that require in-depth knowledge of the database architecture and other technical information. In addition, data generated by projects can become inaccessible after the completion of the project, or left untouched, only to degrade over time. The POPcorn project is addressing these problems by providing a set of search utilities for locating maize projects and Internet resources, BLASTing against multiple sequence targets provided by multiple projects and data warehouses, and for searching sequence-indexed data (i.e., data linked to nucleotide and/or protein sequences). To help prevent data from being lost when projects are completed, POPcorn is now in the process of developing tools such as ZeAlign for migrating project data to MaizeGDB for long-term storage.

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

P22

The new MaizeGDB video tutorial page

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We have developed a new tutorial website, http://outreach.maizegdb.org/, where you can access our video tutorials on topics of interest to maize researchers. This site is available from the MaizeGDB homepage (MaizeGDB.org) with a single click on "MaizeGDB tutorial" in the upper left corner. We have created videos on how to use various features of the MaizeGDB database and interface. In addition, as a community service, we have developed tutorials on the methods and outcomes of large collaborative projects, including how data were generated. The first set of these video tutorials explains how the Maize Genome Sequencing Consortium sequenced and assembled the first reference genome B73, as well as how to best access and understand the data as made available at MaizeGDB. Currently we are working on outreach videos for the Maize Hapmap (Gore et al, Science. 2009 Nov 20;326(5956):1115-7). We strive to make these informative and easy to understand for both beginning students and experienced maize researchers, in order to make new technologies and products more accessible to all. As always, please let us know what types of tutorials you would like to see!

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

P23

MaizeGDB Curation Activities -- Diverse Genomes, Gene Expression, Community GO Annotation

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We report here on the data integration at MaizeGDB for the HAPMAP project of the Maize Diversity Project (Gore *et al.* 2009 *Science* 326:1115-7); and gene expression data from the B73 Gene Atlas Wisconsin 2010 (Sekhon *et al. Plant Journal* in press). Links and interactions with other sites are described. These data are the result of highly improved and relatively inexpensive sequence-based technologies, the use of which we anticipate is likely to expand extensively in the very near future. Handling these data, requested by members of the maize community and encouraged by the MaizeGDB Working Group, will require new strategies for data integration along with appropriate interface development and improvements to current data storage capabilities. Model queries will be posted with the goal to stimulate input by conference attendees. We will also report on a new collaboration with Truman State University to transfer their Gene Ontology-based functional annotation of gene models with published experimental evidence (Buckner *et al.* 2007 *Genetics* 176:741-747).

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P24

MaizeGDB virtualized infrastructure

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Over the past year, the MaizeGDB system infrastructure has been completely redesign and virtualized to insure availability and increase performance. Here we show the overall design of the virtualized infrastructure, our server environment, updated software, and methods to gather usage statistics. Moving to a virtualized environment has had significant impacts on how we do business. We are happy to report that previous physical hardware limitations no longer bog down development, thus improving our ability to provide timely software releases that support your research needs.

Funding acknowledgement: United States Department of Agriculture (USDA)

P25

Insertional Mutagenesis In Maize Using *Ds*: Web Resources and Tools at PlantGDB

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We have developed genetic screens, molecular methods and Web resources for utilizing *Dissociation* (*Ds*) as an insertional mutagen in maize. Over 1700 *Ds* elements have been distributed throughout the maize genome in a uniform genetic background (inbred W22) to serve as donor elements for local or regional mutagenesis. This poster will describe Web-based tools for browsing, searching and accessing these genetic resources at PlantGDB, a website for a comparative plant genomics. Based on *Ds*-flanking sequences that have been provisionally placed in the B73 RefGen_v2 genome assembly, researchers can search for *Ds* insertions close to any gene or chromosomal region using online query tools or BLAST. Other resources include a primer design tool for confirming *Ds* insertion and a request form for ordering seedstocks. The *Ac/Ds*Tagging project pages can be accessed at http://plantgdb.org/prj/AcDsTagging/. **Hands-on workshops to train researchers in using *Ds* lines for reverse genetics are planned for summer 2011 and 2012 in Ithaca, New York and Ames, Iowa, respectively.** The development of these *Ds* insertion lines promises to greatly accelerate functional genomics studies in maize.

Funding acknowledgement: National Science Foundation (NSF)

P26

Who Moved My Exon? Improving Gene Structure Annotations for Maize

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Genome annotation is an iterative process in which computationally-derived structures are incrementally improved over time with additional data, better filters and as errors are detected and corrected. But errors may persist for some time until addressed in a new release, not an ideal situation for researchers. Involvement of the research community in the annotation improvement process is valuable but may be limited by the lack of readily accessible tools and training. There is also a need for methods to organize and divide the annotation effort and for reporting and disseminating progress. We have sought to address these needs through enhancements to the yrGATE gene structure annotation platform at PlantGDB (Wilkerson *et al*., 2006, Genome Biol 7:R58), and we are using the maize genome as a test case for how a community annotation effort could be organized and implemented. Recent enhancements to yrGATE include: updated Web interface and search capabilities; ability to assign annotation groups, group administrators, and projects; standardized metadata to describe annotations; a new Web display that integrates all available data on published loci and dynamically displays yrGATE annotations in progress; and context-sensitive help and tutorials. CpGAT, a new web-based genome annotation tool, is also available to allow users to incorporate new alignment evidence. All curated yrGATE annotations are instantly viewable at MaizeGDB via DAS (Distributed Annotation Service). The poster will summarize initial and ongoing improvements to the maize B73 RefGen_v2 genome / 5a.59 working gene set (from maizesequence.org) using yrGATE. Progress can be tracked at http://plantgdb.org/ZmGDB/DisplayLoci.php?status=all or by Atom feed at http://plantgdb.org/site/yrgate_new_annotations.php?GDB=ZmGDB. Maize researchers are encouraged to create an annotation account and contribute their own re-annotated loci.

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P27

Modulating Nitrogen Flux in Sorghum and Wheat

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Nitrogen assimilation and remobilization is essential in plant growth and development. To maximize crop yield and plant productivity large amounts of nitrogenous fertilizers are often applied to fields. Modulating nitrogen flux to improve nitrogen use efficiency (NUE) would not only reduce input costs but also help mitigate pollution of aquatic ecosystems by the reduction of fertilizer inputs. As a means to perturb nitrogen flux in sorghum (*Sorghum bicolor* (L.) Moench) and wheat (*Triticum aestivum* L.), we have developed a set of transgenic events via *Agrobacterium tumefaciens*-mediated transformation. This set includes expression cassettes that harbor (i) a barley alanine amino transferase (*Hv*ALA-AT), driven by either a rice tissue specific promoter (*Os*Ant1) or the sugarcane *UBI4* promoter; (ii) a rice cytosolic glutamine synthetase (*Os*GS1) driven by the sugarcane *UBI4* promoter; (iii) a rice glutamate synthase (*Os*GOGAT) driven by the sugarcane *UBI4* promoter; and (iv) the maize *DOF1* transcription factor driven by either *UBI4* promoter or the maize *rbcS1* promoter. Preliminary characterization of progeny derived from these expression cassettes in both sorghum and wheat will be presented. Our short-term goal is to identify lead events based on greenhouse phenotype studies for use in down-stream field evaluations.

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P28

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

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The necrotrophic fungal pathogen *Cochliobolus carbonum* race 1 (CCR1) is the casual agent of maize leaf spot and ear mold through the action of HC-toxin, an inhibitor of histone deacetylases. The dominant maize *Hm1* gene encodes HC-toxin reductase (HCTR), a NADPH-dependent enzyme that reduces a ketone group necessary for the toxin's biological activity, thus providing complete resistance to CCR1 at all stages of plant development. Two naturally occurring variants of *Hm1* are *Hm1A*, an allele differing by a single S99Y substitution, and *Hm2*, a duplicate of *Hm1* on a different chromosome encoding a truncated protein. In contrast to *Hm1*, resistance conferred by either *Hm1A* or *Hm2* is age-dependent, with plants exhibiting little resistance for the first four weeks and full protection at maturity. In addition, *Hm2* confers dosage-dependent resistance somewhat weaker than that provided by *Hm1A*. In general, changes in source-sink relationships during development may result in a surplus of photosynthates available for use in stress responses at maturity. Experimental evidence for the same, however, has been lacking till date. Since HCTR activity is dependent on a NADPH cofactor, a correlation between the available levels of NADPH and the expressed degree of resistance to CCR1 pathogen infection with development is thus hypothesized. By overexpressing the protein of each naturally occurring allele, we plan to determine their *in vitro* HCTR activity rates at varying levels of NADPH and HC-toxin. Additionally, we will generate novel alleles through site-directed mutagenesis to determine which residues and domains are necessary and sufficient for HCTR activity. Directly measurements of NADPH levels on a daily basis in plants at different stages of development will be coupled with analysis of disease lesion development at various times of the day and under different light-dark regimes to further elucidate the bioenergetics underlying adult plant resistance.

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P29

A public platform for the verification of the phenotypic effect of candidate genes for resistance to aflatoxin accumulation and *A. flavus* resistance in maize

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Many projects have identified candidate genes for resistance to aflatoxin accumulation or *Aspergillus flavus* resistance in maize using large scale expression or proteomics studies. However, a vanishingly small percentage of these candidates have been validated under field conditions and their relative contribution to resistance, if any, is unknown. The Corn Host Plant Resistance Research Unit has created a candidate gene testing pipeline that consists of steps for identifying, testing, and verifying the association of any maize gene sequence with resistance. The resources associated with the pipeline include: the CFRAS database of DNA and protein sequences associated with past resistance studies (developed in conjunction with Mississippi State University); a diverse panel of 8 inbred lines for SNP identification within any given maize gene sequence (the panel is soon to be fully sequenced, and until it is, any individual gene can be quickly sequenced in the panel as part of the pipeline); four QTL mapping populations and one association mapping panel, (all fully phenotyped over multiple years, locations, and replications for aflatoxin accumulation resistance and associated phenotypes); and capacity for SSR and SNP genotyping in the population(s). Eleven genes identified as possible candidate genes from the database of previous studies were put through the entire candidate gene testing pipeline and results are presented here to illustrate the steps in the pipeline. Polymorphisms based on InDels or SNPs within each gene were identified and were mapped to the correct genomic location in every case. Three genes were found to have a small but significant effect on aflatoxin accumulation resistance in one environment, according to the QTL mapping populations. Any maize gene sequence can be tested in this pipeline, which we hope to do in a collaborative manner for all interested parties.

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P30

An endosperm enzyme catalyzes the formation of phosphotrieser and phosphdiester bonding complex between nucleic acids with altering their stucture

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A newly discovered enzyme, that can catalyzes the alteration of the structure of nucleic acid through the formation of phosphotriester and phosphodiester bonding complex, was partially purified from maize developing endosperms by combining following sequential steps: 15%-35% ammonium sulfate fractionation, DEAE-cellulose anion exchange column chromatography and Sephadex G150 Gel filtration. Endosperms of W64A maize post 22 days pollination was used for this study. Routinely 50 mM of Tris-HCl buffer pH 7.5 was used for preparation of enzyme extract, ammonium sulfate fractionation, DEAE anion exchange column chromatography, except that on DEAE column chromatography, the enzyme was stepwise eluted out from the column by 50 mM, 100 mM, 200 mM sodium chloride included in Tris buffer, respectively. The enzyme activities were monitored by the changing of OD at 260 nm as a result of the formation of a complex of either phosphotriester or phosphodiester bonding between nucleic acids. The optimum pH value for the enzyme activity is in 50 mM acetate buffer at pH 5.4. The enzyme is widely distributed in nature ranging from biological tissues to viral particles including barley mosaic virus, southern bean mosaic virus as well as poliovirus and many others. The broad presence of this enzyme in biological kingdom suggests that the enzyme is an evolution significant protein. A varieties of short and long chain length of nucleotides had been tested for demonstrating the catalysis of enzyme reaction. It was found that the enzyme can exist in both monomer (24,000KD) and dimer (50,000KD) forms on Sephadex G150 gel filtration. Both enzyme forms can catalyze the reaction. Data of 1% agarose gel eletrophoresis in 50 mM Tris-HCl buffer at pH 7.5 from the enzyme reaction products from either short or long chain length of nucleotides as substrates showed consistently staying at the origin of loading well without migration indicate that the enzyme can carry out the linking of multi-nucleotides together through either phosphotriester and/or phosphodiester bonding and the formation of a very large lolecular structure (aggregate polymer form). A possible reaction mechanism of this enzyme and the significant biological function are presented and discussed. This study had been carried out on and off more two decades in the laboratory of the late Dr. Oliver E. Nelson

P31

Basis and Selection for Quality Protein Maize (QPM)

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Quality protein maize (QPM) restored kernel hardness of high lysine *o2* soft endosperm, but two needs remained unmet, our knowledge of the molecular basis of *o2* modifiers (*Mo2*) and an accelerated conversion of any germplasm to facilitate its broader application at reasonable cost. To explore these two needs, a series of RNAi transgenes, blocking γ-, β- and α-zein synthesis, were generated.

Previous studies showed that one of the QTLs was linked to the 27-kDa γ-zein locus on chromosome 7S. Indeed, QPM lines had 2- to 3-fold higher levels of 27-kDa γ-zein. Moreover, it could be shown that elimination of γ-zeins disrupted endosperm modification by *Mo2*, indicating their hypostasis to γ-zeins. Abnormalities in protein body structure and their interaction with starch granules in the F1 with *Mo2*/+; *o2*/*o2*; *γRNAi*/+ genotype suggested that γ-zeins were essential for restoring protein body density and starch grain interaction in QPM.

For conversion of elite lines into QPM, breeders first have to make both parental lines homozygous for *o2*, and then convert them into QPM, respectively. During this process, breeders have to monitor the recessiveness of *o2* and the presence of *Mo2*, a lengthy process that discourages the spread of the benefits of QPM to consumers. On the basis of the hypostasis of γ-zeins, we developed a universal and accelerated QPM conversion approach. Instead of using the recessive *o2* mutation, we were using an RNAi construct against both 22- and 19-kDa zeins, but linked to the visible GFP marker gene. Indeed, when such green and non-vitreous phenotypes were crossed with QPM lines, *Mo2* produces a vitreous green kernel, illustrating that high-lysine and kernel hardness can be selected in a dominant fashion. Furthermore, it then becomes easy to replace the transgene either with *o2* again or a transgene without the GFP.

Funding acknowledgement: Selman A. Waksman Chair in Molecular Genetics at Rutgers University

P32

Characterization of the Expression and Function of Pyrophosphate Dependent Phosphofructokinase (PFP) in Endosperm Maturation in Quality Protein Maize

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PFP is an adaptive plant glycolytic enzyme that uses PPi instead of ATP. When active, PFP exists as a heterotetramer of two catalytic β–subunits and two regulatory α–subunits. Evidence suggests that the α–subunit only accumulates during stressful conditions such as Pi starvation and anoxia that result in reduced availability of ATP but not PPi. We show that PFPα is down-regulated both at the transcript and protein levels in opaque2 (o2) endosperm and strongly up-regulated in QPM endosperm compared with wild type while PFPβ transcript and protein are invariant. PFPα transcript and protein do not differentially accumulate during early endosperm development in support of a role for PFP later, during the manifestation of the opaque2 phenotype. PFPβ transcript and protein remain constant in wild type, o2 and QPM endosperm and embryo. We propose that high-level expression of PFPα is involved in endosperm modification through amelioration of ATP draining cellular stresses that exist in o2 endosperm. We identified two PFPα transcripts that are differentially abundant and virtually identical except that one has a 105 bp region encoding a 35 amino acid exon that is absent in the other transcript. We show evidence that the shorter transcript results from an expressed retrogene. To investigate the assembly of the reported PFP heterotetramer in QPM, we used size exclusion HPLC of native protein extracts to confirm that the heterotetramer only accumulates in QPM but in wild type and o2 endosperm, a dimer exists. Probing these fractions with PFPβ antibody suggests that a homotetramer containing only PFPβ exists in wild type and o2 endosperm. Enzyme assays show an increase in PFP activity in QPM endosperm. To further determine the significance of PFP in QPM, transgenic over-expression and under-expression lines of the two PFPα genes are being recovered.

P33

Control of Granular Starch Accumulation by Starch Synthase III when Isoamylase-type Starch Debranching Enzyme is Compromised

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Starch synthatse III (SSIII) is one of only two enzymes known to condition accumulation of phytoglycogen in maize endosperm, the other being isoamylase-type starch debranching enzyme (ISA). SSIII mutations alone do not cause this so-called "sugary" phenotype, however, when the ISA1 protein is compromised by the *su1-P* mutation, the gene *du1* that encodes SSIII becomes a determining factor for accumulation of semi-crystalline storage glucans as opposed to soluble polymers. In this study SSIII was shown to condition phytoglycogen accumulation also in lines lacking functional ISA2 protein. Endosperm tissue completely missing both SSIII and ISA2 fails to display any ISA activity in zymograms, even though either single defect alone does not eliminate ISA activity or substantially reduce starch content. The ISA homomer complex in the double null mutant assembles and migrates normally in anion exchange chromatography, gel permeation chromatography, and native-PAGE. Thus, SSIII affects the activity of homomeric ISA without influencing it's assembly. Coupling the *du1-Ref* mutation, which produces a low level of a trunctated SSIII protein, with the *isa2*$^{-}$ null mutation also caused a sugary phenotype. In this instance the ISA homomer did display activity in zymograms. These data may be explained by a direct or indirect effect by which SSIII influences the post translational modification of assembled ISA1 homomer and thus affects its enzymatic activity. This activity of SSIII is not required for normal starch accumulation if the ISA1/ISA2 heteromer is present. Taken together the data indicate specific functional connections between SSIII and ISA in the determination of storage glucan architecture.

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P34

Control of cell fate acquisition during maize leaf development

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Using the model crop *Zea mays*, our lab aims to unravel how novel cell types are differentiated along the leaf. Along its length, a mature maize leaf exhibits four distinct cell types: blade, auricle, ligule and sheath. Ligule cells differentiate at the boundary between distal blade and proximal sheath cells; auricle cells arise on the distal side of this boundary after ligule initiation. My work aims to investigate interactions between the few factors known to play a role in this process as well as to uncover novel regulators controlling leaf cell fate acquisition.

The function of two transcription factors are together required for ligule and auricle identity, *ligeuless1* (LG1) and *liguleless2* (LG2). To model the pathway in which LG1 and LG2 act, I am investigating potential physical and genetic interactions between these proteins and two others proposed to function during ligule and auricle development, *Wavy auricle in blade* (WAB) and *liguleless narrow* (LGN).

Additionally, morphological differences in vascular patterning are apparent at the blade/ sheath boundary and the hormone auxin is implicated as a major factor in guiding these patterning events in leaf tissue. *ZmPIN1a* is strongly expressed early during preligule band differentiation suggesting a role for this protein in patterning this structure. To place a LG1/ LG2 pathway within the developmental time course of leaf development, I am investigating whether induction of these genes is downstream of auxin mediated patterning or whether their activation precedes *ZmPIN1a* expression in this domain.

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

P35

Developing a Bacterial Model for a Mitochondrial Defect

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Cytoplasmic male sterility (CMS) is a condition in which anthers do not produce viable pollen due to a mitochondrial defect. In maize (*Zea mays*) CMS-S, recombination between the circular mitochondrial DNA (mtDNA) and linear plasmids leads to linearized forms of the main mitochondrial genome. The transcription of a 1.6-kb RNA, which includes two open reading frames, *orf355/77*, from linear mtDNA ends is associated with sterility. We hypothesize that a "toxin" encoded by one of the two *orf*s is produced during the starch-filling stage of pollen development. To identify which part of *orf355/77* results in toxicity, a bacterial model of CMS-S is being constructed. The 1.6 kb region as well as 1.1 kb and 0.6 kb portions of *orf355/77* were PCR amplified from CMS-S mtDNA. The three DNA fragments were cloned into the pGEM-T Easy vector or the CloneJET vector. Each DNA fragment was amplified from the clones and inserted into the *E.coli* expression system. This approach should allow us to determine if the expression of the 1.6-kb, 1.1-kb or 0.6-kb region is toxic to *E.coli* cells. If any region is associated with toxicity, we will determine if the toxicity is on the RNA or protein level.

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P36

Developing a robust Virus-induced gene silencing (VIGS) system for maize

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VIGS is a widely-used tool to suppress the expression of specific genes. We are using the tripartite Brome Mosaic Virus (BMV) system, originally developed for VIGS in barley and rice. VIGS in maize was also achieved using this system but was somewhat transient and unreliable. We are developing this system and screening multiple maize lines in order to develop a more robust protocol for the routine use of VIGS in maize. Details of our progress will be presented.

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P37

Dosage-dependent genes affecting seed composition or weight

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Kernel quality traits are important targets for grain improvement. Kernel quality is a complex phenotype determined in part by chemical composition and size. We identified maize seed mutants with potentially rate-limiting steps for quality traits. We screened the UniformMu transposon tagging population using a custom-built grain analyzer that collects near-infrared spectra (NIR) and seed weights from individual seeds. NIR spectroscopy is an analytical technique that reports seed composition non-destructively. Our analyzer has 100- to 1,000-fold greater throughput than other single-kernel NIR systems enabling genomics scale analysis of individual seed phenotypes. Using an automated multivariate statistical analysis workflow, 1,012 UniformMu ears segregating for *defective kernel (dek)* phenotypes were screened. NIR/weight data predicted *dek* heterozygous genotypes through multiple generations for 8 mutant isolates. The seed-dosage effect was further confirmed for each mutant through outcrosses to the W22 inbred. We are using next generation sequencing of transposon insertion sites to identify linked insertions to the dosage-effect loci.

P38

Expression Analysis of the Cellulose Synthase (*CesA*) Gene Family Indicates that *CesA10*, *CesA11*, and *CesA12* are Strongly, Though Not Exclusively Associated with Secondary Cell Wall Biosynthesis

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Our overall goal is to enhance understanding of how cell wall constituents are synthesized and integrated, a process central to improving utilization of maize for grain, fiber, and renewable energy. To address this, two approaches are employed here. First, we profile expression of the cellulose synthase (*CesA*) gene family at cell- and tissue-specific levels using qRT-PCR. We also characterize *CesA* expression in suspension cells undergoing hormonally-induced secondary cell wall deposition. In addition to the known *CesA* family members, we also measure expression of a bioinformatically identified *CesA7* paralog (*CesA7P*). Tissue-specific mRNA levels were measured at three developmental phases; seedling emergence, vegetative growth, and anthesis. Expression levels in kernel tissues were also assessed. The *CesA10*, *CesA11*, and *CesA12* family members were consistently expressed in similar patterns. Other *CesA's* grouped together based on expression patterns across tissues, and these changed throughout development. At a given stage of development, most family members could be placed in subgroups that shared overall expression patterns to varying degrees. Some family members, however, were expressed independently of all others. The striking similarity in expression patterns of *CesA10*, *CesA11*, and *CesA12*, and extent of secondary wall formation at sites of maximal expression indicates that these genes are strongly, although not exclusively, involved in secondary cell wall biosynthesis. Furthermore, these genes are highly upregulated in suspension cells that have been induced to produce tracheary element-like secondary cell walls. Collectively, the expression profiles are consistent with previous hypotheses that different CESA's may function in diverse heterohexameric complexes.

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P39

Flavonoid mediated resistance to anthracnose leaf blight in sorghum and transfer of this trait into maize

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Anthracnonse leaf blight (ALB) caused by *Colletotrichum sublineolum* is one of the most devastating diseases of sorghum. Area under sorghum cultivation in the US is rapidly increasing as it is an ideal feedstock for biofuel production. Losses due to this disease are anticipated to increase in importance with widespread cultivation. The disease inoculum survives in the field debris and can repeatedly infect the plant at any stage during its life cycle. Although disease incidence can be reduced by tilling and rotating with non-cereal crops, utilizing the innate mechanisms of disease resistance is more promising. One option is the 3-deoxyanthocyanidins, a class of compounds that are produced in response to fungal attack and have antifungal properties. Studies have shown that the more resistant cultivars of sorghum are able to induce these compounds more rapidly and intensely. The biosynthesis of these compounds is regulated by a Myb transcription factor *yellow seed 1* (*y1*). Herein we show that a functional *y1* gene is required for 3-deoxyanthocyanidin mediated resistance in sorghum. Maize, a close relative of sorghum; is able to produce numerous flavonoid compounds under the regulation of *pericarp color 1* (*p1*). However, none of these compounds are as fungi-toxic as the 3-deoxyanthocyanidins and are not rapidly induced under fungal infection. We are attempting to identify maize germplasm that can produce 3-deoxyanthocyanidins to enhance resistance to ALB which also occurs in maize. The sorghum *y1* gene has been introduced into maize in an effort to engineer the biosynthesis of antifungal compounds. We have characterized the response of maize lines transformed with the reporter construct and native sorghum *y1* gene.

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P40

Genetic Control of Starch Digestion: Better Food & Fuel

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Starch from domesticated cereals is a fundamental part of most human activity. The starch is typically broken down to glucose for biochemical energy from food and, more recently, to serve as feedstock for fermented biofuels. However, these two uses demand opposite characteristics of cereal starch. The health crisis posed by obesity, diabetes, and related disease, as well as the need for more efficiently utilized livestock feed call for slow, steady glucose release from digesting maize, while glucose release for biofuel should be rapid to minimize energy inputs and costs. Combining near-infrared spectroscopy and digestion assays we have identified mutants in the maize TILLING population that represent four, altered starch digestion types from crude flour: rapidly digesting when cooked, rapidly digesting when uncooked, slowly digesting, and high glucose yield. We are taking a combined, QTL, starch structure and endosperm proteomics approach to understanding what genes and gene products are altered in these mutants.

Funding acknowledgement: Consortium for Plant Biotechnology Research (CPBR), Syngenta

P41

Genetic and physiological characterization of a wilted *Zea mays* mutant

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Characterizing root water stress mutants helps us understand key factors influencing plant water relations. We observed individuals with slow root growth rates under osmotic stress within an F2 family derived from an EMS mutagenized B73 parent. In the field, the same family segregated for a wilted phenotype. Stem cross sections of the mutant plants showed normally developed metaxylem elements suggesting water transport was not compromised. An F2 B73 x B73-wilted population segregated for the wilted trait in the field but did not exhibit the mutant phenotype under greenhouse conditions. The ratio of mutant and wild type plants from an F2 A619 x B73-wilted population in the field studies was 1:3 in one year and lower in a second year. Thus, the penetrance of the wilted phenotype is dependent on the environment. A scan of genome wide SNP markers identified several markers on the long arm of chromosome 7 associated with the mutant phenotype. Fine mapping and physiological characterization is ongoing.

P42

Genetic basis of adult plant resistance in maize to a lethal leaf blight and ear mold pathogen

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The pathosystem involving maize and Cochliobolus carbonum race 1 (CCR1), the causal agent of a lethal leaf blight and ear mold disease, has a number of interesting features. This includes the presence of a number of disease resistance genes/alleles that confer protection in a developmentally specified manner. In contrast to the key wild type gene of this pathosystem - the famous Hm1 gene, which keeps every part of the plant immune to CCR1 at every stage of development, many of the developmentally specified genes confer effective protection only when maize plants reach maturity. Cloning of two of such adult plant resistance (APR) genes has shown that while the protein encoded by one of these (Hm1A) differs from HM1 by a single amino acid, the protein of the other (Hm2) is truncated and lacks the last 52 amino acids present in HM1. These findings indicated that the APR behavior of Hm1A and Hm2 was due to their partial rather than complete loss-of-function, which is to reduce HC-toxin produced by CCR1. To obtain a genetic proof for this hypothesis, we generated new APR alleles of Hm1 (the wild-type gene) by directed EMS mutagenesis. Sequencing of these APR alleles has validated the hypothesis that the APR nature of the disease resistance genes in the maize-CCR1 pathosystem is the result of their compromised activity.

P43

Genome-wide association study of carbon and nitrogen metabolism in the maize nested association mapping population

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Carbon and nitrogen metabolism is critical to plant growth and development. We evaluated the maize nested association mapping population for quantitative variation of 12 carbon and nitrogen metabolites. In total, about 12,000 samples and more than 100,000 assays were processed with the robotized platform. Using joint linkage analyses, common QTL were identified for different metabolites especially for those with related pathways. We found that the correlations between different metabolites are driven by the proportion of shared QTL. Therefore, we were able to build up the relationships of the studied metabolites based either on their correlations or proportion of shared QTL. Using genome wide associate study (GWAS), we identified SNPs from different candidate genes associated with various metabolites. For example, in one case, we identified SNPs from *carbonic anhydrase* genes associated with multiple different metabolites in nitrogen metabolism and photosynthesis pathways; in another case, SNPs from *invertase* genes associated with metabolites from starch synthesis pathway. We demonstrated here that joint linkage analysis combined with GWAS in maize NAM population can be an efficient way to dissect natural variation in conserved metabolic pathways.

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P44

Identification of herbivore-induced transcription factors involved in the terpene biosynthesis in maize

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The production of volatiles, especially terpenes, is essential for the indirect defense of plants. Maize attacked aboveground by the lepidopteran larvae *Spodoptera littoralis* and belowground by the larvae of the coleopteran *Diabrotica virgifera virgifera*, emit different blends of volatiles which consist mostly of mono- and sesquiterpenes. These volatiles can attract natural enemies of both herbivores which are parasitic wasps aboveground and entomophatogenic nematodes belowground. Although the enzymes responsible for the production of these volatiles have been identified [1, 2], little is known about the signal transduction pathways between herbivore damage and volatile production.

To elucidate the signal transduction pathways in roots and leaves, we conducted a transcriptome analysis in maize within four hours after herbivory. We identified several groups of transcription factors and other regulatory factors that are induced at different times after herbivory. While some of the factors were solely induced by mechanical wounding of the plant (Mecworm), others required the presence of insect-derived elicitors. The results indicate multiple parallel signal transduction pathways of which some are dependent on a jasmonic acid intermediate.

[1] Köllner T.G.; *et al*., Plant Cell, 20; (2008), 482-49
[2] Schnee C.; *et al*, PNAS, 106; (2003), 1129-1134

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P45

Interactions with growth media affect the efficacy of a potent brassinosteroid biosynthesis inhibitor in maize

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An important goal of modern agriculture is to minimize production inputs with simultaneous maximizing of yields. A strategy to strengthen plant architecture and optimize the use of water and fertilizer is the employment of dwarf and semi-dwarf crop varieties especially in wheat and rice. Growth inhibitors have been used extensively to study the underlying mechanisms of height regulation in plants.
Substrates used to grow plants can have a major effect on the efficacy of growth regulators. These interactions were investigated by root zone application of differing concentrations of the brassinosteroid biosynthesis inhibitor propiconazole (PCZ) on maize seedlings. In addition to testing multiple media types under differing PCZ concentrations, media mixes of Turface with standard media. Pre-germinated maize seedlings were treated with PCZ and the reduction of cell elongation of mesocotyls was measured. Experiments were performed with light-grown (greenhouse) plants, and in dark-grown (growth chambers) plants to induce etiolation. The lightly expanded clay aggregate (LECA) substrate Turface had a major inhibitory effect on efficacy of PCZ. Other substrates such as Vermiculite and Perlite did not interfere with PCZ activity. We will present the effect of PCZ treatments on maize seedlings on different growth substrates as well as quantification of PCZ after differing time exposure to the growth substrates to assess their binding capacity and mode of inactivation for PCZ.

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P46

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 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 are being 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. This project was funded by grant NSF DBI-0701405.

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P47

Maize *opaque5* Encodes Monogalactosyldiacylglycerol Synthase and Specifically Affects $C_{18:3}/C_{18:2}$ Galactolipids Necessary for Amyloplast and Chloroplast Function

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The maize *opaque5* (*o5*) locus was shown to encode the monogalactosyldiacylglycerol synthase MGD1. Mutations of *o5* affect the vitreous nature of mature endosperm and chlorophyll accumulation in leaves. An allelic series engendered stepwise reductions in gene function and $C_{18:3}/C_{18:2}$ galactolipid abundance relative to $C_{18:3}/C_{18:3}$ species was reduced proportionally, without significant effects on total galactolipid content. This alteration in polar lipid composition disrupted the organization of thylakoid membranes. Mutant endosperms showed altered frequency of glucan chains that form crystalline lamellae of starch granules, reduced rate of granule initiation, and enlarged mature starch grains. Assembly of isoamylase-type starch debranching enzyme and an unidentified amylase were altered in mutant endosperm. The null allele caused kernel lethality owing to failure in both endosperm and embryo development. Starch assembly in null mutant endosperm was altered such that the regular arrangement of growth rings typical of normal granules was abolished. The data demonstrate that low abundance galactolipids with five double bonds serve functions in plastid membranes that are not replaced by the predominant species with six double bonds. These functions in amyloplast membranes affect starch granule formation. The results also suggest that MGD1 can distinguish the constituency of acyl groups on its diacylglycerol substrate.

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P48

Maize aldose reductase: A role in sugar-handling?

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Our initial interest in aldose reductase (AR) rose from its possible role in sorbitol metabolism by maize kernels. This is the only known enzyme in maize, other than sorbitol dehydrogenase, with capacity to synthesize or use the sorbitol so prominent in developing kernels. The reaction is reversible (sorbitol+NADP<=>glucose+NADP[H]) and could allow sorbitol use by embryos. However, ARs can catalyze diverse reactions and may have multiple roles in sugar- and redox-handling. Aldo-keto reductases (AKRs) are widely distributed in nature and play numerous roles in metabolism. In this study, we present eight maize putative AKRs and characterize one of them, AKR4C13, due to its embryo specificity. To analyze in detail the expression of maize AKRs at the protein level we raised polyclonal antisera against the recombinant maize AR and used it in western blot assays. We also designed specific primers for each of the eight putative maize ARs to analyze their gene expression in different tissues and development times. The analysis of different maize tissues showed reaction with several polypeptides. The amount of each polypeptide also appeared to vary among tissues, consistent with potentially different roles for the AR-like polypeptides. Data on western blots were consistent with predicted molecular weights of the AR family members as well as their expression patterns. The AKR4C13 was embryo-specific, with a MW of 35,659 Da and was temporally correlated with seed maturation. Analysis of the recombinant AKR4C13 enzyme indicated that, in addition to DL-glyceraldehyde, reactions favored use of NADPH to reduce pentoses, but not D-glucose. Maize AKR4C13 was also able to oxidize sorbitol in the presence of NADP. One possibility is that the maize sorbitol pathway, and AR in vivo, has similarities to roles in humans, where their primary effect is that of balancing sugar pools and redox levels under high-sugar conditions.

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P49

Monitoring Ds Transposition in Soybean Genome

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The maize two-component transposon system *Ac/Ds* has been used in many plant species as a means to generate insertional and activation tagged mutants. The long-term goal of this program is to develop a repository of transgenic soybean events harboring mapped *Ds* elements positioned approximately every cM, thereby creating a collection of *Ds* soybean events that will have utility for local mutagenesis. The usefulness of the system will be influenced by the ability of *Ds* to transpose when stacked with *Ac*. To investigate the transposition of *Ds* in the soybean genome we selected a set of five soybean events harboring gene or enhancer trap elements delineated by *Ds* termini. These events carry either one or two transgenic loci. To induce transposition we stacked the respective *Ds* events with an *Ac* cassette under control of either the constitutive 35S CaMV promoter or the reported meiosis specific promoter, DMC1, from Arabidopsis and generated 351 crosses. We found somatic transpositions in 144 F1 plants and 14 germinal transpositions in the progeny of 23 F1 plants analyzed so far. One specific transposition has a *Ds* delineated enhancer trap element re-inserted into the third intron of the ligand gated potassium ion channel (glyma 06g08110). In addition we have generated 461 events carrying an activation tag construct delineated by *Ds* termini and crossed selected events with the *Ac* carrying events to generate another 121 crosses. The data gathered from this study will allow us to test the influence of both level, and tissue specificity of *Ac* expression on transposition of *Ds* in the soybean genome.

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P50

Moving towards efficient, reliable and high throughput double haploid line development

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Hybrid breeding exploits heterosis exhibited by the combination of genomes from two inbred lines. In maize, inbred lines are traditionally produced by 7-9 generations of selfing. Doubled- haploid production has emerged as a viable alternative to traditional inbred development. Production of double haploid lines involves pollen-mediated induction of gynogenetic haploids followed by chromosome doubling. The advantage of this technique over the conventional method is that 100% homozygous inbred lines are obtained in just two generations. Despite the advantages, some technical issues limit the use of this technique. These issues include poor induction capabilities of inducer lines, poor adaptation of inducer lines to maize growing regions of the U.S.A and developing countries, difficulties in identification of haploid seed and the use of inefficient, toxic and labor-intensive doubling methods. We have initiated a suite of experiments to address some of these issues. Several alternative inducer lines with later flowering, improved drought and heat stress resistance, and pest resistance are being developed. We have evaluated several microtubule binding herbicides as less toxic alternatives to colchicine, as well as different methods of delivery. Using flow cytometric analysis of nuclear DNA content in expanded leaves we have been able to rapidly compare doubling agents and treatment methods. In addition, implementation of an embryo rescue protocol has allowed the recovery of seedlings in 30d less time than mature seed scoring while preserving efficient scoring of the R-nj allele used to distinguish haploids from diploids. Mapping populations are currently being developed to identify QTLs contributing to the induction capabilities of the inducer and permit the introgression of further improvements to induction, flowering time and stress adaptation.

Funding acknowledgement: Purdue University Agricultural Research Programs

P51

Nucleosome distribution and promoter architecture at 400 genes in the maize genome

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In maize cells, more than four meters of DNA is organized within a nucleus that is approximately five micrometers in diameter. The cell is able to compensate for this length of DNA by packaging the DNA into chromatin. The fundamental subunit of chromatin is the nucleosome, which is approximately 150 basepairs of DNA wrapped about 1.6 times around a histone octamer core. This organization into chromatin, affects all DNA-templated processes. We assume that nucleosome distribution plays a role in regulating all nuclear processes (transcription, replication, recombination and repair), but this assumption is largely untested as there are few large data sets describing nucleosme position in eukaryotic cells. To address this gap in our description of the maize genome, we have designed a high density tiling microarray containing two kilobases surrounding the transcription start sites of each of 400 genes from the recently sequenced maize genome. We have prepared nuclei from multiple maize tissues, cut these nuclei with the internucleosmal cleavage agent micrococcal nuclease, and prepared the mononucleosomally protected DNA to probe our high density tiling microarray. We will identify promoter architectures consistent with known models chromatin regulation (poised transcription start sites consisting of a nucleosome free region flanked by two positioned nucleosomes), as well as unique locus specific promoter architectures. This work provides the first insights into the chromatin structural regulatory control of hundreds of maize genes. We expect that these descriptions of the links between chromatin structure remodeling and nucleosome position will provide a platform for future studies of chromatin structure in maize and other eukaryotic species.

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P52

Role of carbohydrate metabolism on the functioning of lodicules during the opening of staminate flowers in maize

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The male flowers in maize tassel have a unique and complex structure called floret, which consists of carpels and stamens, and several special lateral organs (glumes, lodicules, lemma and palea). Lodicules in particular are organs considered to be homologous to petals in eudicots, and play a role in the floret opening process. In the male florets, the lodicules are two diminutive bodies that are lying between the lemma and the ovary base and expand rapidly at the time of anthesis. They accomplish their role by levering away the rigid glumes, thus allowing anthers and stigmas to emerge. Several physiological studies have established the role of environmental and endogenous factors controlling the events of anthesis. However, very little information has been published regarding the metabolism and the role of carbohydrates inside the lodicules during the swelling process. We analyzed enzymatic activities and sugar and starch concentrations during lodicule swelling through the floret opening process of B73 plants. We divided the floret opening in seven stages. Lodicules were dissected to run the metabolite analyses and to determine relative water content and fresh and dry mass. In general, the process includes water influx and metabolite storage in both lodicules and filaments. The opening process starts one day before the anthesis, in which the "ready to open florets" separate from the inflorescence rachis. In these florets, we detected that a water flux and metabolite storage to the lodicules starts at stage 2, when the glumes separate apart from each other. Maximum water flux is reached just at anthesis when the lodicules have the highest size and water content. Kinetics of the lodicule fresh and dry mass showed that during the swelling process these organs grow 2.5 times more as compared to its initial size. In general, sucrose and starch contents were low and constant through the swelling process; however, dramatic changes were observed in fructose and glucose. Our data suggests that carbohydrate metabolism inside the lodicules occurs during the anthesis process, in which not sucrose, but glucose seems to play an important role as the osmolite responsible for lodicule swelling and floret opening.

P53

Structural Diversity among Maize Haplotypes

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Maize exhibits levels of structural variation (SV) of non-repeat sequences that are unprecedented among higher eukaryotes. This SV includes hundreds of copy number variants (CNVs) and thousands of presence/absence variants (PAVs). Many of the PAVs contain intact, expressed, single-copy genes that are present in one haplotype but absent from another. Array-based comparative genome hybridization (CGH) experiments has identified genes that missing in some inbreds relative to the B73 reference genome (*Springer et al., 2009, PLoS Genetics*). Whole Genome Shotgun (WGS) re-sequencing of non-reference inbreds has identified genes that are missing from the reference genome (*Lai et al., 2010, Nature Genetics*). The "Zeanome", a near-complete set of genes present in B73, the NAM founders and teosinte, is being defined using existing genomic sequence data and newly generated transcriptomic data. By assembling transcriptomic sequences and comparing the resulting contigs to the B73 reference genome hundreds of expressed sequences that are missing from the B73 reference genome were identified. These genic PAVs may contribute to the phenotypic diversity of this important crop.

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P54

Structure-function analysis of the maize NLR1 protein, a plant-specific nuclear-localized protein

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The flat leaf blade of higher plants is an important adaptation for efficient photosynthesis. We recovered a maize mutant that impacts development in multiple tissues including distinct *narrow leaf and rough endosperm (nlr1)* phenotypes. We identified a *Robertson's Mutator (Mu)* transposon insertion tightly linked to nlr1. The transposon disrupts the coding sequence of a J-domain containing protein leading to reduced accumulation of the full length transcript. J-domains activate Hsp70 ATPase and proteins containing J-domains have diverse functions in the cell. In addition to the J-domain, the protein contains two nuclear localization signals (NLS) and an Arginine/Serine (RS)-rich domain at the N-terminus. RS domains are found in pre-mRNA splicing factors and presence of this domain suggests NLR1 is associated with spliceosomal complexes. Transient expression of N and C terminal fusions with GFP show subnuclear localization consistent with nuclear speckles. pre-mRNA splicing factors are frequently localized to nuclear speckles. Domain deletion assays revealed that the N-terminal RS domain is required for the speckling pattern, while deletion of either the C-terminal NLS or J-Domain has no affect on the localization pattern. A yeast-two-hybrid (Y2H) screen using NLR1 as bait, retrieved FK506-binding protein 12 (FKBP12). Members of the FKBP family are immunophilins and possess peptidyl-prolyl cis/trans isomerase activity. FKBPs are ubiquitous and serve in protein folding, cell stress, signal transduction, transcription and cell cycle regulation. Arabidopsis FKBP12 has roles throughout plant development, especially during endosperm development and embryogenesis. Interestingly, AtFKBP12 is known to interact with AtFIP37, which is homologous to HsWTAP and DmFL, two metazoan proteins involved in splicing. These data support a model in which NLR1 is involved in transcriptional regulation through interaction with spliceosomal complexes.

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P55

Study of a viviparous mutant impaired in the last phase of the ABA pathway

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*vp*404* is a viviparous mutant with light green seedlings, reduced chlorophyll and carotenoids content and lower ABA level in both embryo and seedling tissue when compared to wild-type. When the mutant is exposed to decreasing values of relative humidity the stomata conductivity is not altered while transpiration is enhanced with a rapid decrease in seedling weight. Addiction of ABA (10^{-7}molL^{-1}) to the MS medium restores wild-type values. When immature embryos (25 DAP) are grown on solidified MS medium the mutants elongate more than the wild-type siblings, showing less sensitivity to the hormone. These responses are expected if the mutant is impaired in ABA biosynthetic pathway. The results of the complementation test with all known *vp* mutants with green seedling indicates that *vp*404* defines a new vp gene. *vp10* and *vp15* control the last step of ABA biosynthesis. Crosses of the *vp*404* mutant with TB-10L and TB-5L, uncovering *vp10* and *vp15* respectively, gave a negative result. These two mutations affect genes *vp10* and *vp15* encoding the proteins (CNX1 and respectively CNX7) required for the Molybdenum cofactor (MoCo) pathway. MoCo is a cofactor required for the activity of Abscissic Aldehyde Oxidase (AAO), involved in the last step of ABA biosynthesis, and also for Nitrate Reductase (NR), Sulphite Oxidase (SO), Xanthine Dehydrogenase (XDH). Four other *Zmcnx* genes (encoding CNX proteins) are known to regulate MoCo biosynthesis. To verify the possibility that *vp*404* could be a mutation of one of these genes, we are analysing the activity of AAO, directly involved in ABA biosynthesis as well as the other enzymes requiring MoCo (NR, SO, XDH). These analyses will be done using spectrophotometric and native electrophoresis tests, and the results will be reported.

P56

The cuticle of maize silks as a model system to define the pathway of hydrocarbon biosynthesis

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Simple non-isoprenoid hydrocarbons (e.g., alkanes and alkenes) are major components in petroleum but are found only in discrete parts of the biosphere. Extant biological hydrocarbons are relatively abundant in the cuticle of plants and insects, and function to create a water barrier for these organisms. Although the biological production of hydrocarbons most likely occurs via the conversion of fatty acids to alkanes and alkenes, the biochemical mechanism of this conversion is not defined. The cuticle of maize silk is largely comprised of linear hydrocarbons (lengths from C_{23} to C_{33}) and is the most abundant source of hydrocarbons in maize. The pathway for hydrocarbon synthesis is developmentally regulated; silks that have emerged from ear husks contain ~5-fold higher concentrations of hydrocarbons as compared to silks still encased by the husks. As a first step toward identifying QTL responsible for this trait, we have demonstrated that the hydrocarbon load on emerged silks varies as much as ~6-fold across the 26 diverse founder lines used in the Nested Association Mapping (NAM) population. In addition, the distribution of individual hydrocarbon constituents significantly differs among inbreds (e.g., the C_{33} hydrocarbon varied from 10% to 68% of total hydrocarbon content in the Tx303 and Ms71 inbreds). A complementary RNA-seq approach is currently underway to identify differentially expressed genes within the transcriptomes of silks that have emerged, as compared to those still encased within the ear husks. Together, these approaches are the first steps toward defining the genetic and biochemical pathways responsible for the production of hydrocarbons, which could have a significant impact on the production of biofuels.

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P57

The molecular genetic dissection of C4 traits in maize and *Setaria viridis*, model systems for C4 biology

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C4 crops outperform C3 crops under hot, dry conditions; they have reduced photorespiration and greater water and nitrogen use efficiencies. These gains are achieved through intra- or intercellular compartmentalization of photosynthetic activities. Two major innovations in C4 photosynthesis include enhanced metabolic exchange between two photosynthetic cell types, the bundle sheath (BS) and the mesophyll (M) and a suberized BS cell wall to limit CO2 diffusion. The biosynthesis of suberin monomers has been partially characterized in Arabidopsis roots and potato periderm. However, little is known about the function, biosynthesis, and transcriptional regulation of BS suberization in C4 species. We identified a subset of genes that are expressed during BS suberin deposition in maize and assembled a putative biosynthetic pathway based on functional characterizations from *A. thaliana* and *S. tuberosum*. To elucidate the functional role of BS suberization in C4 species, we are currently targeting several biosynthetic enzymes in reverse genetic screens using the maize Ac/Ds transposons and through an RNAi-based approach using the new C4 model system *Setaria viridis*. We are also characterizing two dicarboxylate transporters, DiT1 and DiT2 that likely play critical roles in the movement of malate between BS and M cells in C4 plants. However, the mechanisms that control their cell-specific expression in maize are unknown. We used comparative genomic analysis to identify putative cis-regulatory elements. We intend to characterize the network of trans-factors that may regulate the expression of DiT1 and DiT2, using CNS::GUS transgenic lines, yeast one- hybrid assays and ChIP-seq. The characterization of key features of the C4 syndrome will be highly useful for the development of novel crop plants that will meet the world's growing food, feed, and energy needs.

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P58

The stem as a dynamic carbohydrate reservoir in maize

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There are several factors that affect the development, growth and yield of maize plants. Most physiological, biochemical and molecular studies have been focused on different organs, among those, the stem is the least studied in maize. The stem is usually understood only as a structural support and a static channel through which high concentrations of sucrose and other nutrients pass from the source leaves to the sinks tissues. We challenged this simple view by hypothesizing that the stem besides having a static function is also a dynamic reservoir of non-structural carbohydrates (NSC) that is affected by conditions such as stage of development, water stress and by removal of the source or the sink. We performed several experiments with different subtropical maize genotypes. In all, the whole stems were sampled, its juice was extracted and starch, glucose, fructose and sucrose were measured. There were highly significant differences of stem NSC in response to the different treatments. Some effects were also genotype dependent. The WS treatment increased soluble sugars but decreased starch in comparison to the well watered (WW) regime. The levels of sucrose increased exponentially during development, whereas glucose and fructose had a transitory peak at the stage of female flowering. Removal of the sink lead to a strong increase of stem starch, whereas removal of the source had only subtle effects on stem NSC. A very active metabolism in the stem parenchyma seems to modulate the hexose/sucrose ratio and starch. Based on these and other results, we conclude that stem NSC are neither a static nor a simple reflection of sucrose transport through the phloem. Therefore, we suggest that, instead of being regarded as a boring tube with a support and transport function, the maize stem should be rather regarded as a dynamic carbohydrate reservoir that modulates the supply of sugars to other tissues.

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P59

Using the Corngrass1 gene to enhance the biofuel properties of crop plants

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All land plants undergo dramatic developmental changes during the juvenile to adult phase transition. In general, juvenile plant material is less lignified and display differences in biomass character and accumulation. Thus, by identifying and controlling the genes that specify this transition, it may be possible to enhance the biomass properties of any crop plant of choice.

Our analysis of the dominant Corngrass1 (Cg1) mutant in maize has directed us to a group of plant specific transcription factors that controls this phase transition. Cg1 mutant plants are fixed in the juvenile phase of development and increase biomass of vegetative shoots by continuously initiating axillary meristems and juvenile leaves. Furthermore, Cg1 leaves contain decreased amounts of lignin and increased levels of glucose and other sugars. Thus, the Cg1 gene keeps the maize plant in a prolonged juvenile state, causing increased biomass and providing an improved substrate for fermentation.

We cloned Cg1 and showed that it is an unusual grass-specific tandem microRNA gene that is overexpressed in the mutant. Since the target genes of this microRNA are highly conserved in many plant species, we hypothesized that it should be possible to transfer the biofuel properties of the maize Cg1 mutant into any crop of choice simply by overexpressing the Cg1 cDNA and downregulating its targets. This was tested in the model dicot Arabidopsis, the model tree Populus, the model grass system Brachypodium, and the biofuel crop plant Panicum virgatum (switchgrass).

Field trials of transgenic switchgrass plants overexpressing the maize Cg1 gene were completed last summer. Similar to maize Cg1 mutants, these plants displayed increased vegetative biomass and dramatic alterations in flowering time. Moreover, composition analysis using FTIR microscopy and NMR showed that overall lignin content was reduced, the ratio of glucans to xylans was increased, and surprisingly, that starch levels were greatly increased. These results point to the utility of this approach for designing new biofuel crop plants.

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P60

ZmCIPK9, a maize CBL-interacting protein is involved in the signaling pathway of potassium uptake in plant

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Recent studies have shown that a set of calcium sensors named Calcineurin B-like proteins (CBLs) and their target proteins named CBL interacting protein kinases (CIPKs) form a complex signaling network and mediate plant responses to a variety of external stresses. Recently we have identified 10 CBLs and ~40 CIPKs in maize. Here we report that one of the maize CIPK genes, ZmCIPK9 is involved in the signaling pathway of potassium uptake in plant, which encodes a polypeptide of 448 amino acids. Database analysis revealed that the deduced amino acids has high similarity with Arabidopsis AtCIPK23. The transgenic experiment showed that overexpression of ZmCIPK9 in Arabidopsis mutant cipk23 could completely rescue its sensitivity phenotype under low potassium conditions. We further found that the interaction of ZmCIPK9 with Arabidopsis CBL1 and CBL9 by yeast two-hybrid analysis. Moreover, yeast two-hybrid screening with 10 maize CBLs showed that ZmCIPK9 could interact with ZmCBL1, ZmCBL4 and ZmCBL9. Subcellular localization demonstrated that ZmCIPK9 is distributed in the nucleus, plasma membrane and cytoplasm, and the kinase analysis showed that the auto phosphorylation of ZmCIPK9 in the presence of Mg2+. Taken together, our results provide evidence for involvement of a maize CIPK gene, ZmCIPK9 in potassium uptake in response to low potassium stress conditions.

P61

necrotic upper tips1 is responsible for proper water movement during the floral phase of development

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Maize is very sensitive to water stress, especially during the floral transition. Insufficient water transport during this time can have severe detrimental effects that manifest as leaf wilting, tassel browning, and sterility, which together comprise a condition known as "tassel blasting." Special mechanisms must exist to ensure sufficient water transport during this critical period in order to prevent tassel blasting from occurring. We have identified a mutant from an Activtor (Ac) transposon screen, necrotic upper tips1 (nut1), which mimics tassel blasting. The nut1 mutant phenotype is evident only after the floral transition, while early vegetative development is normal. nut1 upper stems and leaves have difficulty moving water as shown by dye uptake and movement assays. Plastic sections and TEM of nut1 vasculature show defects in xylem vessel integrity, which could provide the basis for its mutant phenotype. Double mutants of nut1 and a mutant that produces extra tassel branches, ramosa2 (ra2), greatly enhance the nut1 phenotype, indicating that tassel branches require extra water movement for proper growth. The nut1 mutant is caused by an Ac insertion into the transcription unit of a NAM-like transcription factor. Wildtype revertants were isolated with restored open reading frames caused by Ac excision, thus proving that loss of this transcription factor is responsible for the nut1 phenotype. In situ hybridization of nut1 transcript showed localization to the procambium and early xylem vessels. These results show that unique transcription factors function during the floral phase to help maintain xylem vessel integrity during periods of high water movement in maize.

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P62

Recent advances in Paramutation in Maize

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Paramutation is a heritable change in gene expression induced by allele interactions. It was discovered in maize by Alexander Brink in 1950s when he observed heritable changes in the expression of particular r1 alleles after heterozygosity with other specific r1 alleles. DNA's little cousin RNA is needed for the intriguing gene interactions known as paramutation. Paramutation doesn't follow the laws of classical mendelian genetics. In maize, paramutation has been described for four genes (r1,b1,pl1 and p1) all of which encode transcription factors that activate the biosynthesis of flavonoid pigments in plant or seed tissue .
Two models have been proposed to explain the trans communication that occurs during paramutation- the pairing model and the trans RNA model. Paramutation resembles a genetic mutation in that it is heritable change but differs from it in its high frequency, potential reversibility and non-random occurrence. Paramutation doesn't cause a change in DNA sequence but rather a change in DNA methylation and chromatin structure, and is therefore a classical example of an epigenetic modification.
It is provides an excellent system for studying mechanisms involved in establishing and maintaining heritable expression states and allelic communication. Potential roles of paramutation include important cellular mechanism for protection against invasive DNA, association with recombination mechanism, establishment and maintenance of chromatin domain boundaries and adaptive mechanism for transmission of gene expression states.

P63

Plant Phenotype Dosage Effects of Maize Simple B-A Translocations in a W22 Inbred Background

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Central to genetic research is the effect that genes have on phenotype. Phenotypes may be changed by varying the gene dosage. By utilizing the B-A translocation's ability to undergo nondisjunction during the second pollen mitosis, we have changed the dosage of the genes located on the A segment. Plants which contain one copy (hypoploid), two copies (diploid) or three copies (hyperploid) of their respective A chromosomal segments were produced in an inbred W22 background for 14 of the 20 maize chromosomal arms. The hypoploid and hyperploid plants were compared to the diploid plants in respect to their leaf length, leaf width, plant height, ear height, internode length, stalk circumference, tassel-branch number, days to anthesis, days to silk emergence, and days between anthesis and silk emergence. We will present evidence for significant deviations of hypoploid and hyperploid phenotype values from the diploid values for those chromosome segments that exert strong dosage effects.

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P64

A New Variant of Maize Abnormal Chromosome 10 Confirms Independent Neocentromere Activity of Two Knob Repeats

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Knobs are very prevalent in all Zea species and have been observed at 34 loci in domesticated maize. They are composed of long tandem arrays of two repetitive DNA sequences, TR-1 and knob 180. The largest knob is found on a structural variant of chromosome 10, called abnormal chromosome 10 (Ab10). Ab10 is fascinating because it is able to preferentially transmit itself over a normal chromosome 10 during female meiosis. Moreover, every chromosome that is heterozygous for knob size is also preferentially transmitted in Ab10s presence. The working model for this meiotic drive phenomenon is that it requires neocentromere activity. When Ab10 is present all knobs are transformed into neocentromeres that actively move poleward during meiotic anaphase. Here we describe a new variant of Ab10, Ab10-III, which is probably similar to the structure previously known as K10L2. Interestingly, Ab10-III has neocentromere activity of only the TR-1 repeat, confirming previous evidence that the neocentromere activities of the knob 180 and TR-1 repeats are controlled by independent proteins. Preliminary tests suggest that Ab10-III also displays weak preferential segregation. We are conducting studies to further characterize the behavior of Ab10-III compared to the other two Ab10 variants. Additionally, we are investigating the abundance of Ab10-III in landrace populations via molecular markers and fluorescence in situ hybridization (FISH).

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P65

A spontaneous compensating translocation permits recovery of a telomere-truncated chromosome in maize

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Telomere-mediated truncation constructs have been used to engineer minichromosomes in maize by truncation of endogenous chromosomes and recovery of centric fragments (Yu et al., 2006; 2007). Maize minichromosomes were easily recovered from B chromosomes, which lack known genes, but were rarely recovered from A chromosomes whose truncation leads to deficiency gametes. The only mini-A chromosome recovered in previous studies was derived from a spontaneous tetraploid embryo culture (Yu et al., 2006), which contained extra doses of the truncated DNA and masked the deficiency. In this study we transformed maize embryos using telomere-truncation constructs, and detected a T_0 plant containing a large truncation of the short arm of chromosome 1 (*trunc-1*) and a *de novo* compensating translocation chromosome (*super-6*). *Super-6* was composed of the truncated fragment of chromosome 1S that had rearranged with one copy of chromosome 6, permitting recovery of *trunc-1*. The transgene signal localized near the end of the broken chromosome arm on *trunc-1*. *Trunc-1* and *super-6* were transmitted together as a heterozygote to ~41% - 55% of progeny. Transmission of *trunc-1* as an addition chromosome occurred in ~15% of progeny. Reciprocal crosses to a tester plant revealed that neither chromosome transmitted through pollen. Transgene expression (Bar) cosegregated with trunc-1 transcriptionally and phenotypically. Analysis of meiosis in T_1 plants revealed 8 bivalents and one tetravalent chain composed of chromosomes 1, *trunc-1*, 6, and *super-6* in diplonema and diakinesis. The data suggests that the *de novo* compensating translocation allowed recovery of the truncated A chromosome by compensating the deficiency in female gametes and by altering chromosome pairing and segregation. The truncated chromosome can be maintained as an extra chromosome or as a heterozygote with *super-6*. These data indicate that spontaneous mutations that occur during telomere-mediated truncation can involve the formation of compensating translocations capable of masking the deficiency.

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P66

Centromere specific sequences change and centromere reactivation in newly formed chromosomes in maize

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During screening the progeny of chromosome B-9-Dp-9 undergoing the BFB cycle, we found a A-B translocation chromosome named 9 Bic-1, in which the B centromere was translocated to the short arm of chromosome 9 and inactivated. Though the B centromere of 9 Bic-1 is inactive, the 9-Bic-1 chromosome can undergo nondisjunction when an intact B chromosome is added in the same background. Due to nondisjunction, the short arm of 9 Bic-1 will be broken at the second pollen mitosis and produce new chromosomes with different breakpoints. In the progeny, we found 9S-Bic-1 and 9S-9S-Bic-1. Chromosome 9S-Bic-1 was broken in the A centromere, which contains centric repeats CentC and CRM, and an active A centromere. Chromosome 9S-9S-Bic-1 was from 9 Bic-1, breaking in the A centromere and forming as an isochromosome. It is interesting to note that its A centromere lost detectable CentC sequences but is still active, and the two B centromeres are inactive.
In the progeny of 9S-9S-Bic-1 plants, we found several rearranged chromosomes. One newly formed chromosome contained two B centromeres derived from 9S-9S-Bic-1, but one centromere has centromere function. In this case, reactivation of an inactive centromere from 9-Bic-1 was found. The new chromosome has three centromere regions, which are rearranged in their orientation. A functional A centromere without CentC and CRM has also been found in the progeny of 9-Bic-1. It is surprising to find that the functional B centromere can lose the CentC and CRM. In the next generation of the 9S-Bic-1 chromosome, we also found ZmBs were eliminated and this new variant chromosome is stable and transmitted to progeny.

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P67

Characterization of *mtm99-14* and *mtm99-25*; meiotic mutants with defects in homologous pairing

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Meiosis is a highly conserved process in eukaryotes that is essential for producing haploid gametes with genetic variation. During meiotic prophase, homologous chromosomes find each other, pair, synapse and undergo homologous recombination. During synapsis, an axial element assembles between the two sister chromatids of each homolog and the central element assembles between the axial/lateral elements of the two homologous chromosomes to form a tripartite synaptonemal complex (SC). Here, we present two mutants with defects in homologous pairing. By TEM, spread prophase chromosomes of *mtm99-14* display irregular and thicker axial elements than wild-type chromosomes. AFD1 and ASY1, axial element components, are assembled onto chromosomes with the same timing and distribution as in wild-type. However, ZYP1, a component of the central element, starts loading onto the chromosomes during zygotene, but fails to complete assembly resulting in the formation of large patches of ZYP1 in addition to elongated band structures. In pachytene, ZYP1 elongation is delayed between homologously as well as non-homologously paired chromosomes of *mtm99-14*. These results suggest that *mtm99-14* is defective in synapsis due to anomalous formation of the axial element and/or their maturation into the lateral element as the SC forms during zygotene.
A failure of homologous pairing resulting in dramatic non-homologous synapsis including multiple exchanges of synapsis partners and numerous foldbacks are an important feature of the *mtm99-25* phenotype. However, ASY1, AFD1 and ZYP1, components of the SC, are loaded and assembled as in wild-type. Moreover, a tripartite SC is observed in both homologously and non-homologously synapsed chromosomes by TEM and immunolocalization. At the leptozene/zygotene transition and zygotene, the heterochromatic knobs in wt elongate, however, in *mtm99-25* knob elongation is more extensive and persistent. These observations suggest that regulation of heterochromatin morphology by *mtm99-25* is essential for homologous pairing to occur normally. Using map based cloning and high throughput sequencing methods, we have mapped *mtm99-25* to Chr. 5 and have a candidate gene for *mtm99-14*. .

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P68

Competition of Different Sized Centromeres as an Examination of the Centromere Drive Hypothesis

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Meiotic drive can be a powerful selective force in evolution. Different sized centromeres have been hypothesized to be able to cause an unequal selection of one chromosome over another for transmission to the egg pole during meiosis (meiotic drive). A portion of the centromeric histone, CenH3, is rapidly changing during evolution. It is hypothesized that this is in response to the self promoting elements in centromeres. CenH3 may be coevolving to negate the drive of the selfish centromere elements. This hypothesis is difficult to test under normal circumstances because there is seldom size variation between centromeres of homologous chromosomes. TB-9Sb is a reciprocal translocation between the short arm of chromosome 9 and the B chromosome. B-9S is the chromosome with a B centromere and the short arm of 9, while the 9B chromosome has the majority of chromosome 9 and a piece of the B chromosome translocated onto the short arm and shows a sequence of B specific repeat at the tip of the translocation. To test the hypothesis that self-promoting elements in the centromere create meiotic drive, we used normal TB-9Sb, and four TB-9Sb derived lines having the B-9S centromeres reduced in size by misdivision. To test the transmission ratios, crosses were made between TB-9Sb and each misdivision derivative; the progeny of these crosses were screened to find plants with one TB-9Sb and one misdivision derivative. The selected progeny were then pollinated by c sh wx or HiII so that the progeny plants will have a normal chromosome 9, one or the other B-9S and one 9S-B. These progeny are screened for the presence of the normal or reduced sized centromere B-9S. Our data thus far has shown no statistically significant selection for either centromere. These data provide no obvious evidence for centromere drive based on the size of the centromere, but we note that even a slight selection for one centromere would quickly compound over successive generations and lead to fixation in a population. However, centromere size seems an unlikely determinant of meiotic drive.

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P69

Construction Of A Cytogenetic Map Of Maize In Oat Addition Lines Using Sorghum Bacterial Artificial Chromosomes (BACs) As Fluorescent Probes

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We are developing a pachytene cytogenetic map of the maize (*Zea mays* L.) genome by performing Fluorescence *in situ* Hybridization (FISH) of maize marker-selected sorghum bacterial artificial chromosomes (BACs). Our objectives are to cytogenetically map the core bin markers (CBM) located 10μM apart on the pachytene chromosomes 1, 3, 4, 5, 6, & 8 and to FISH map two regions duplicated between chromosome 9 with chromosomes 1 (9-1) and 6 (9-6). Several techniques that involve different chromosomal substrates and treatments as well as various probe selection and labeling methods are available. This project uses the techniques described in Figueroa *et al.* (2011) as well as publicly available resources for maize, oat, and sorghum. Briefly, the maize chromosomes, each isolated in their own maize addition line of oat, are optically isolated by genomic *in situ* hybridization (GISH) with direct-labeled total maize DNA. The centromere is identified using a direct-labeled centromere-specific repeat sequence, Cent-C. Maize CBM-RFLPs are used to identify sorghum BACs with homologous sequences, which are then used as representative FISH probes. FISH data for chromosomes 1, 4, 5, and 6 is presented along with data of FISH mapped loci duplicated between chromosomes 1 and 9 using a single BAC. These results facilitate analysis of the maize and sorghum genomes by using common markers to integrate their physical, linkage, and cytogenetic pachytene chromosome maps and will allow us to determine if the maize genome sequence is an adequate predictor of physical location on the maize pachytene chromosomes. The maize cytogenetic FISH map is described at cytomaize.org and mapping records as well as FISH images are made available at MaizeGDB.

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P70

Endoreduplication of a Very Small Telomere-Truncated Minichromosome Derived From the B Chromosome

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Endoreduplication is the replication of a chromosome in which the cell progresses through multiple synthesis phases without performing mitosis, resulting in multiple copies of the genome in a single cell. This phenomenon typically occurs as a natural process in terminally differentiated cells, but can be present in all cell types as a consequence of a breakdown in normal chromosomal processes. Here we describe a minichromosome that regularly endoreduplicates. The minichromosome was initially a very small chromosome consisting of the B chromosome centromere, transgenes, and very little if any adjacent chromatin. While in the process of increasing the copy number of this minichromosome mitotic instabilities became obvious, as the copy numbers fluctuated from as low as zero to as high as eight in a single root tip. More endoreduplication seems to occur as the copy number fluctuates within a root tip, indicated by large increases in the size of minichromosomes. The endoreduplication is evident not only by the size, but also by the increased numbers of centromere and transgene FISH signals found on these chromosomes. The larger endoreduplicants have many centromere signals in contrast to the progenitor chromosome. In meiosis, smaller endoreduplicants will faithfully segregate more often than their larger counterparts, which usually lag or proceed to one pole or the other.

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P71

Genetic variation in the meiotic recombination pathway in maize

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Meiotic recombination is a major source of genetic variation in plants. Although the role of recombination in evolution is recognized, little is known about how evolutionary forces affect the recombination pathway itself. Theoretical predictions and empirical studies suggest that changes in the meiotic recombination pathway may provide adaptive abilities to populations experiencing directional or strong selection pressures, such as those occurring during species domestication. To examine how maize evolution and domestication affected meiotic recombination genes, we studied evolution patterns in eleven genes controlling key recombination pathway steps in a diverse set of maize inbred lines and several teosinte accessions. Even though meiotic recombination genes generally exhibit high sequence conservation expected in a pathway controlling a key cellular process, we identified footprints of various evolution modes. In a number of analyzed genes, we found signatures of adaptive evolution. Relatively few amino acid residues showed polymorphism among the maize inbreds and teosinte accessions. Through protein structure predictions, we found that several of these amino acid residues were likely to induce changes in the protein function. We also analyzed crossover frequencies in a number of maize inbred lines and found that, although the overall extent of variation was relatively low, there were numerous statistically significant differences. We propose that evolutionary changes in the recombination pathway may have contributed to the successful domestication of maize and its expansion to new cultivation areas.

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P72

Identification and Characterization of a Population in situ of Perennial Teosinte found in Ziracuarétiro, Michoacán, Mexico

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Teosinte populations in situ in southern parts of Mexico were monitored for collection and conservation ex situ during 2008-9. The collection mission resulted in a new perennial teosinte population (accession T-162, CIMMYT Acc. ID 29739) located at Fresno (1380m, 19°25':00″N, 101°:54':40″W), Ziracuarétiro, Michoacán, Mexico. Cytological analysis of T-162 has confirmed that the teosinte population belongs to Zea perennis because it has perennial plants; is a tetraploid with 2n=40; and pachytene chromosomes show terminal small knobs. In order to identify it in the known phylogeny of Zea, 12 teosinte accessions of the known species and 2 maize lines were genotyped together with the new teosinte population (CIMMYT acc. ID 29739). 1,165 SNPs with good quality were used for phylogenetic, diversity and principal component analysis. The perennial teosintes including Fresno population (T-162) from Ziracuarétiro, and two Z. diploperennis populations from Las Joyas (T-39) and San Miguel (T-43) in the Sierra de Manantlán, Jalisco formed a clade that was supported by a bootstrap value of 100%. However, T-162 branched separate from T-39 and T-43 in a Neighbor-Joining tree containing 15 accessions, confirming it is a different taxon from Z. diploperennis. Phylogenic relationship of other species of the genus Zea in the study was the same as the results of previous reports. Phylogenetic clustering showed good agreement with the result of principal component analysis. The Fresno perennial teosinte population of Ziracuarétiro, Michoacán is the third population of Z. perennis found in Mexico after currently endangered population of Piedra Ancha, Cidudad Guzmán at an altitude of 2100m and the extinct population, one mile south of the rail way station of Ciudad Guzman. It seems adapted in subtropical environment and much more robust than the population of Piedra Ancha in situ. The new Z. perennis population is used as forage by a farmer who maintains it along the borders of slash-burn maize plots.

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P73

Identification of two mutations (*smd3* and *smd12*) that abolish neocentromere activity at 180bp knob repeats but not TR1 repeats

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Ab10 (Abnormal Chromosome 10) is a rare version of chromosome 10 associated with meiotic drive where, in the female parent, Ab10 is preferentially segregated into approximately 70% of the offspring. We have carried out multiple screens for mutants of meiotic drive in active Robertson's Mutator lines. Previously, only two non-deletion mutations have been characterized, smd1 and smd3. Here we report the discovery of three additional mutants – *smd8*, *smd12*, and *smd13* – which, like *smd3*, are totally defective for meiotic drive. Recent analyses reveal that *smd3*, *smd12* and *smd13* are specifically defective for only one form of neocentromere activity associated with the major 180 bp knob repeat. The fact that TR1-mediated neocentromere activity is retained in these mutants helps to explain several confusing observations we have made in the past. We now believe that 180 bp neocentromere activity is absolutely required for meiotic drive, and that TR1 activity alone, while producing visible neocentromeres, is not sufficient to confer drive. These and other data suggest that the meiotic drive system is actually simpler than we once thought. A critical function we previously referred to as the "distal tip function" is likely to be the 180 bp neocentromere gene (presumably *smd3*, which may be an allele of *smd12* and *smd13*). With multiple mutants of this key drive function we are optimistic about the possibility of characterizing the gene(s) that confer meiotic drive in maize.

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P74

Increasing the copy number of minichromosomes derived from the B chromosome

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Supernumerary B chromosomes possess an array of useful properties for genetic engineering, such as being basically inert and exhibiting an accumulation mechanism consisting of nondisjunction at the second pollen mitosis followed by preferential fertilization of the embryo by the B containing sperm. Minichromosomes, the product of telomere truncation of a maize B chromosome, have lost the nondisjunction property, because the tip of the B long arm required for this function is lost. However, this property can be restored to the minichromosome in the presence of a normal B chromosome. Using FISH, we have initiated a program of minichromosome accumulation. We are testing the accumulation limits of different sizes of minichromosomes with and without transgenes. With a GUS gene incorporated in the telomere truncation construct, we will be able to determine whether there is an onset of silencing at high copy number by analyzing the number of minichromosomes present in a plant. If silencing occurs, we will attempt to lower the number of minichromosomes to elicit re-expression. Minichromosomes will also be used to test the biological limits of chromosome number in plant cells. In maize, about 15 B chromosomes can be accumulated without affecting vigor, which could be from dosage effects of the B on A chromosomes or large increases in chromatin that do not contribute to cellular constituents. Minichromosomes have significantly less chromatin than a normal B chromosome, so saturating the spindle or metaphase plate may be a more significant issue. To date, the largest transgenic minichromosome 1/2B has reached a number of nineteen minis; the next smallest 1/5B is at twelve, and the smallest transgenic mini 1/16B has endoreduplicated after reaching five copies. The two nontransgenic minichromosomes are both near the size of the 1/16B and have accumulated to seventeen minis (mini 20) and nine minis (mini 9).

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P75

Maize whole chromosome painting

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Whole chromosome painting with Fluorescence in Situ Hybridization (FISH), which allows the identification of specific chromosome regions at the DNA level, is an extremely useful technique for comparative studies of plant genome evolution and for detecting chromosome aberrations and rearrangements. Because of the abundance of repetitive elements in the maize genome (up to 80%), selection of chromosome-specific sequences and depletion of repetitive elements from the probe collection are necessary for developing whole chromosome paint FISH probes. Chromosome 8 repeat- free sequences were selected from publically available BAC clones to produce pooled PCR FISH probes. They represented only 0.07% of the chromosome length but were sufficient to paint the whole mitotic chromosome or its arms separately. Related sequences were successfully used by NimbleGen for chromosome-specific array design and probe synthesis. The recently available collection of maize exons (B73 RefGen_1.0) allowed NimbleGen to increase drastically the chromosome coverage with repeat- free sequences and thus increase the resolution and sensitivity of FISH analysis. After masking most repeats and homologous sequences, about 49% of all predicted exons, or about 10% of the total chromosome length, were selected to develop probe pools for chromosomes 1, 5 and 8. The synthetic FISH probes were applied in two colors to study maize chromosome translocations and paint chromosomes of maize relative *Tripsacum dactyloides*. For multicolor karyotyping, development of different labeling schemes of the chromosome-specific probe sets is in progress.

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P76

Microscopic Analysis of Transgenic Maize Lines Expressing a Fluorescent Histone, H2B::mCherry

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Our laboratory recently obtained transgenic lines of maize that were designed to create auto-fluorescent chromosomes. These lines will be broadly useful for microscopic analysis of chromosome behavior in mitosis and meiosis. A gene fusion consisting of monomeric red fluorescent protein (mCherry) and maize histone H2B under the control of a ubiquitin promoter was inserted into the maize genome. Resulting transgenic lines were characterized using PCR and microscopy. Formaldehyde-fixed plant tissues were stained with DAPI and imaged via three - dimensional deconvolution microscopy. We found multiple transformation events that showed strong stable expression in multiple, different somatic tissues, even after prolonged storage. Lines in which the transgene was detected via PCR showed red-fluorescent nuclei, confirming the expected expression of the transgene and localization of the protein in the nucleus. Examination of mitotic metaphase cells further indicated that the protein was stably incorporated into the chromatin. The H2B::mCherry was also found to be stably incorporated throughout all of meiosis I and II, and post-meiotic male gametophyte cells. The mid-prophase pachytene-stage images of histone H2B::mCherry were exceptionally clear and detailed compared to DAPI images. To our knowledge, these findings represent the first known imaging of fluorescent histone protein in maize meiotic nuclei. This material may be ideal for live meiotic imaging. These stable expressing lines represent valuable new reagents for study of chromatin structure, nuclear architecture, and chromosomal behavior in a model plant species with a large complex genome.

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P77

Mitochondrial DNA Sequences in the Nuclear Genomes of Diverse Maize Lines

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The incorporation of mitochondrial DNA (mtDNA) into nuclear genomes continues to occur in flowering plants. We have examined the mtDNA insertion sites within maize nuclear chromosomes (NUMTs) via in fluorescence *in situ* hybridization (Lough et al. Genetics 2008). MtDNA from the NB genome cloned into cosmids was used as probes onto metaphase chromosomes from root tips. The size and distribution of mtDNA insertions varies among maize inbred lines, accounting for some of the chromosomal diversity within maize. In B73, one large insertion on chromosome 9L was shown to contain a majority of the NB mitochondrial genome (Lough et al. Genetics 2008). An examination of a subset of the "diversity lines" of maize (Yu et al. Genetics 2008) is being conducted. Large NUMTs similar to the one reported in B73 have been found on 9L in Oh7B, a non-stiff stalk line, and HP301, a popcorn line. A much smaller insertion of mtDNA, containing only a small part of the mitochondrial genome, exists at this location in many other inbreds. We hypothesized that the small amounts of mtDNA are remnants of an older insertion created early in maize evolution. We have examined a total of 10 inbred lines and two teosinte subspecies (*Zea mays* ssp. *parviglumis* and *mexicana*) for the presence of the pieces of mtDNA shared by the all the previously analyzed lines. We also assessed these lines for the presence of two non-NB mtDNA pieces (2.4 kb and 3.3 kb long) found within overlapping BACs at the B73 9L location. These non-NB mtDNA pieces match the sequence of other Zea mitochondrial genomes, including *Zea mays* ssp. *parviglumis*.

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P78

Studying the meiotic telomere bouquet in maize using the *pam1* mutant

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Meiosis is a process of two successive nuclear divisions producing four haploid gametes, which is essential for sexual reproduction. At the beginning of meiosis, telomeres of all chromosomes attach to the nuclear envelope and cluster, forming a “telomere bouquet”. It is generally believed that the telomere clustering facilitates pairing of homologous chromosomes by bringing their ends together. The bouquet formation is best understood in fission and budding yeasts. However, very little is known about the function of the bouquet in plants. In the past few years our lab developed new cell biology tools to study the function of the bouquet in plants, most notably a technique to monitor chromosome movement in live maize meiocytes. We are also taking a genetic approach to investigate the bouquet function by studying *pam1 (plural abnormalities of meiosis 1)*, a maize mutant defective in meiotic telomere clustering. We found that *pam1* shows significantly decreased chromosome motility in early meiotic prophase compared to wild-type meiocytes. Our previous investigations showed that both the actin and tubulin cytoskeletons are required for the chromosome movements. However, we have not observed obvious cytoskeleton defects in the *pam1* mutant, suggesting that the *Pam1* gene does not control cytoskeleton formation but likely rather affects the link between chromosomes and the cytoplasmic cytoskeleton. In addition, our data show that clustering of nuclear pores, which coincides with the bouquet formation, is largely normal the *pam1* mutant. These data suggest that *Pam1* specifically controls meiotic chromosome motility and the bouquet but not the overall reorganization of the nucleus that takes place in early meiosis.

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P79

The evolution of Kinetochore size

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Kinetochores are the protein structures assembled at the centromeres where spindle fibers attach to during cell divisions to move chromosomes. It has been noticed for years that kinetochores vary in size across species and therefore is assumed that larger chromosomes require larger kinetochores. However, there is little or no correlation between chromosome size and the size of its associated kinetochore, either within species or among species. Furthermore, quantitative measurement of centromeric satellite repeats revealed that kinetochore size is not correlated with the size of centromeric DNA either. To investigate what is responsible for the apparent variation of kinetochore size across species, we measured kinetochore size in Zea mays (maize) and Zea luxurians, two closely related species diverge only 300,000 years ago. We found that in Zea luxurians the kinetochores match the genome size by being 50% larger overall and also roughly 50% larger on a kinetochore-by-kinetochore basis. We then broadened our analysis to the grass family including rice, sorghum, maize, wheat, etc, which vary from 14 chromosomes to 42 chromosomes, and by 40-fold in genome size. These measurements revealed that kinetochore size can be predicted by measuring genome size and dividing by chromosome number. Our data suggest that the large centromeres in most grasses do not reflect a mechanistic requirement to pull large chromosomes, but rather is a reflection of how much kinetochore area is required to stabilize the spindles during mitosis and meiosis. A corollary is that much of the centromeric DNA in plants in not necessary for chromosome segregation.

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P80

The root hair proteome of the maize inbred line B73

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Root hairs are unicellular extensions of specialized epidermis cells on all root types of maize. They substantially increase the root surface area and thereby contribute to water and nutrient uptake. In this study we present a reference proteome for root hairs of the inbred line B73.

To define the maize root hair proteome, label-free Gel LC MS/MS was used to identify 2,573 proteins covered by at least two peptides. Blasting against NCBI.nr and other databases, using Mapman Mercator software resulted in a functional classification for 96% of these proteins. A blastp search was performed to identify homologs of proteins involved in root hair development. In total, 39 maize homologs of 17 proteins previously associated with root hair formation were identified including RTH3 (Hochholdinger *et al*. 2008).

Recently, a root hair reference proteome dataset of the 1,492 most abundant proteins of the dicot model species soybean (*Glycine max*) was published (Brechenmacher *et al*. 2009). In total, 898 homologous proteins were present in both datasets. Detailed functional examination of the soybean and maize root hair proteome datasets suggests conserved, but also unique characteristics of root hairs in dicot and monocot model plants.

(1) Hochholdinger F. *et al*. 2008. The maize (*Zea mays* L.) roothairless3 gene encodes a putative GPI-anchored, monocot-specific, COBRA-like protein that significantly affects grain yield. Plant J. 54: 888-898
(2) Brechenmacher L. *et al*. 2009. Establishment of a protein reference map for soybean root hair cells. Plant Physiol 149: 670-682

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P81

fuzzy tassel encodes a *DICER-LIKE1* protein and is required for vegetative and inflorescence development

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microRNAs (miRNAs) are small, non-coding RNAs that repress gene expression in all multicellular organisms and function in diverse processes including development, stress responses and carcinogenesis. During development, miRNAs are part of regulatory networks that control cell fate and patterning events in both plants and animals. Here, we report the cloning and characterization of the maize *fuzzy tassel (fzt)* mutant, which has dramatic effects on both vegetative and inflorescence development. *fzt* plants are shorter in stature and make fewer, narrower leaves than normal siblings. In addition, inflorescence development is severely perturbed in *fzt*. All meristem types in the inflorescence are abnormal and less determinate than normal; neither the tassel nor the ear makes the normal complement of floral organs and *fzt* is both male and female sterile. We cloned *fzt* using a positional cloning approach and found that it harbors a mutation in the first RNase III domain of DICER-LIKE1, a key enzyme in the miRNA biogenesis pathway. We are currently characterizing the *fzt* vegetative and inflorescence phenotypes in greater detail as well as investigating the effect of the *fzt* mutation on small RNA populations.

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P82

A semi-dominant tassel seed on chromosome 7 of maize affects spikelet meristem determinacy in both the tassel and ear

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Maize produces two types of inflorescences, the tassel and ear. The tassel forms directly from the shoot apical meristem and produces male florets bearing pollen. The ear develops from meristems in the axil of leaves and produces female florets with prominent silks. The developing tassel and ear produce a series of lateral meristems called spikelet pair meristem, spikelet meristems and floral meristem, with determinate fates. Here we present the phenotypic characterization and mapping of a semi-dominant tassel seed mutant first identified in a W22 UniformMu population. After introgressing into B73 and Mo17 the phenotype was notably stronger in Mo17 than in either W22 or B73. Mo17 plants homozygous for the mutation form seeds within tassel spikelets after proliferating a number of bracts and carpel-like floral organs. Ears of the same plants initiate spikelets, but show a proliferation of indeterminate meristems within each glume pair, and never produce seeds. Ears on plants heterozygous for the mutation produce a reduced number of seeds relative to wild type, and many show a reverse germ orientation. Tassels from the same plants produce spikelets with extra male florets. Quantitative analysis of mutants in homozygotes showed significant reduction in tassel length, lateral branch number, central spikelet length, and spikelet pair density on the central spikelet compared to normal siblings. Scanning electron microscopy (SEM) shows the mutant proliferating a series of spikelets within the glume pair of the original spikelet. Fine mapping uncovers a candidate *AP2* -like gene belonging to *AP2* super family of transcription factors that has been implicated in a wide range of plant developmental roles. We find an in-frame 11 amino acid deletion within the first exon of this *AP2/EREBP* gene. RT-PCR analyses indicate that this *AP2/EREBP* gene is expressed strongly in developing tassels and ears. *in situ* hybridization indicates specific expression in inflorescence meristems, spikelet pair meristems, glum primodia as well as spikelet meristems. This semi-dominant mutation results in fertile carpel development in the tassel and indeterminate growth within the spikelet meristem, and may be caused by the misexpression or lack of turnover of an *AP2/EREBP* gene.

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P83

A targeted re-sequencing approach to identify the mutation underlying a fasciated ear mutant

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Meristems must precisely balance the renewal of stem cells with the incorporation of daughter cells into lateral organ primordia. An imbalance in stem cell homeostasis in the inflorescence meristem can lead to a fasciated ear and tassel phenotype. We discovered a mutant we call *fea1905* in an M2 screen of an EMS mutagenized A619 inbred population. We mapped *fea1905* to a 2.7Mb region of chromosome 6 that contains approximately 30 annotated genes.

While DNA sequencing technology has progressed at an impressive rate, it is still not cost-effective to sequence a maize genome to high coverage and quality. Targeted re-sequencing technologies may be used to more efficiently utilize sequencing capacity to cover a target region of the genome. We are attempting to identify the causative mutation underlying the *fea1905* phenotype by genomic hybrid selection on custom-printed Agilent 244k arrays followed by Illumina sequencing. Gene models from the maize working gene set from within a ~5Mb region of chromosome 6 were tiled with 60bp oligos at an offset of 3bp per probe. The probes cover all exons and introns, as well as 1kb upstream and downstream of the gene models. Preliminary results suggest that the amount of DNA captured is limited and not suitable for sequencing, so optimization continues.

In parallel, we are performing mRNA-seq on the mutant and wild-type A619 in an effort to find the mutation in a transcript originating from within the mapping interval. Once the gene is cloned, future work will focus on integrating *fea1905* into existing pathways regulating meristem size in maize.

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P84

A tissue-specific RNA interference strategy to study the role of Arabidopsis Minichromosome Instability 12 (MIS12)

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The kinetochore is a large protein complex that assembles at centromeres to ensure proper chromosome alignment and segregation during cell division. In humans and maize, the MIS12 protein is localized to a central position of the kinetochore serving as a bridge between the inner kinetochore proteins (DNA binding) and the outer kinetochore proteins (microtubule binding). AtMIS12 is constitutively localized to the centromere. Analysis of a T-DNA insertion line indicates that AtMIS12 is essential for cell division. In order to further investigate the function of AtMIS12 and other essential kinetochore genes, we have developed a tissue-specific RNA interference (psRNAi) system. Petals are good targets for tissue-specific RNAi because they are large, visible organs that are not required for growth or reproduction. A portion of the Arabidopsis PISTILLATA (PI) promoter confers early petal-specific expression. We found that when this domain was used to drive a GUS RNAi transgene, GUS expression was knocked down specifically in petals. We have also tested this novel psRNAi system to knockdown the expression of AtMIS12. Plant lines expressing the PI:MIS12 RNAi transgene exhibit an array of defective petal phenotypes. The petal phenotype has proven to be heritable and stable in the T1 and T2 generations. Significant decreases in petal length and significant increases in cell size correspond well to the measured levels of the PI:MIS12 RNAi transgene. Cytological analyses revealed that the petal defects are caused by cell cycle arrest at a prometaphase-like stage. More detailed studies combining FISH and immunolocalization will be used to determine whether the psMIS12 RNAi lines fail during metaphase alignment or anaphase onset. We hope to further use the system to study the pathway of kinetochore assembly and the role of the spindle checkpoint in organogenesis.

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P85

Addressing the involvement of microRNAs in the reprogramming of leaf growth under drought stress in grasses

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Drought is one of the most important environmental factor leading to yield reduction due to growth retardation.

Brachypodium distachyon (Bd), a diploid wild grass whose genome has been recently sequenced, possesses all the properties (e.g. short life cycle and limited growth requirements) that can be desired of a model system for studying the molecular mechanisms involved in plant response to drought stress in grasses. As a drought tolerant grass, originating in Iraq, Bd may possess specific adaptations or tolerance mechanisms which may be transferred to related grass crops.

We have established a soil-based assay, in which we subject the reference accession Bd21 to growth-limiting, non-lethal drought stress. In this condition, drought stress has no effect on cell division rates but affects cell expansion resulting, overall, in a reduced leaf size. We used this effect to address the roles of microRNAs (miRNAs) within the molecular networks controlling leaf size and drought response in temperate grasses.

From two biological replicates we produced four smallRNA libraries obtained from expanding and proliferating leaf zones subjected to drought stress and control conditions.

Using next-generation sequencing techniques the libraries were sequenced. Nearly 30 million reads were analyzed for each library, of which 70-80% perfectly map onto the Bd genome sequence. Our bioinformatics pipeline revealed both conserved and species specific miRNAs families modulating their expression under stress and showing fluctuation between the two developmental areas.

In addition we predicted target genes for these miRNAs molecules to investigate their possible involvement in leaf development in normal and stressed conditions.

P86

Analysis of *stunter1*, a Maize Mutant with Reduced Gametophyte Size and Maternal Effects on Seed Development

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stunter1 (*stt1*) is a novel, recessive, maternal effect mutant in maize that displays viable, miniature kernels. Maternal inheritance of *stt1* results in seeds with reduced but otherwise normal endosperms and embryos. The *stt1* mutation displays reduced transmission through the male and female parents and causes significant changes in the sizes of both male and female gametophytes. *stt1* pollen grains are smaller than wild type, have reduced germination efficiency, and reduced pollen tube growth. *stt1* embryo sacs have smaller central cells and abnormal antipodal cells that are larger, more vacuolated, and fewer in number than wild type. Embryos and endosperms produced by fertilization of *stt1* embryo sacs develop and grow more slowly than wild type. The data suggest that the morphology of mutant embryo sacs influences endosperm development, leading to the production of miniature kernels in *stt1*. Analysis of seeds carrying a mutant maternal allele of *stt1* over a deletion of the paternal allele demonstrates that both parental alleles are active after fertilization in the endosperm and embryo. This analysis also indicates that embryo development until the globular stage in maize can proceed without endosperm development and is likely supported directly by the diploid mother plant.

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P87

Analysis of small RNA-controlled gene networks in SAM function

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The shoot apical meristem (SAM) initiates all above-ground organs of the plant such as leaves. Molecular genetic analyses allowed the identification of key regulatory genes involved in meristem indeterminacy and organ initiation. A number of these genes are under the control of microRNAs, such as HD-ZIP III transcription factors involved in establishment of leaf polarity in maize (Juarez et al, 2004). Each newly formed leaf becomes polarized and develops distinct adaxial (top) and abaxial (bottom) sides. The establishment of adaxial-abaxial polarity requires a complex genetic network involving a cascade of small RNAs, including *miR390, tasiR-ARF* and *miR166* (Noguiera et al, 2007; 2009). To gain insight into small RNA-controlled gene networks required for SAM function and organogenesis, we are characterizing the precise expression patterns of selected mature miRNAs by in situ hybridization and use a focused laser to microdissect regions of interest within the SAM and developing leaves to analyze the expression profiles of the small RNA precursors. Six microRNAs was first analysed (*miR394, miR528, miR160, miR167, miR164* and *miR319*). Each showed a distinct expression pattern suggesting diverse contributions of small RNAs and the pathways they target to the regulation of SAM function. Towards the second goal, 12 distinct regions of the shoot apex, including leaf primordia P0 to P3, the tunica layer and corpus from the SAM, the abaxial and adaxial sides of young primordia, the SAM tip, and stem tissues, were each collected separately. The quality of RNA isolated from these tissue samples has been verified using known marker genes. A first expression analysis of microRNA precursor genes revealed a complex regulation underlies the generation of distinct expression patterns for the mature microRNAs. These RNA samples will also be analyzed by deep sequencing to compare the global gene expression profiles of these distinct cell populations.

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P88

Analysis of the ZmHK1 gene family and its possible roles in leaf patterning

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We have initiated a thorough analysis of the maize semi-dominant mutation, *Hairy Sheath Frayed-1* (*Hsf1*) to determine the effects of hormone signaling on the formation of proximal-distal maize leaf growth patterns. We have cloned the gene underlying the *Hsf1* mutation, *Zea mays Histidine Kinase1* (*ZmHK1*), which encodes a known cytokinin signaling protein. We hypothesize that cytokinin signaling and its proper signal transduction is required for normal maize leaf patterning. We performed phylogenetic analysis using the recently sequenced B73 maize genome sequence as reference and show that the *ZmHK* gene family consists of several additional paralogs than originally reported. We will present results detailing the expression patterns of *ZmHK1* using semi-quantitative RT-PCR and in situ hybridization. In order to understand the mechanism of the *Hsf1* mutation, we have assayed the mutant protein in-vivo using a yeast–cytokinin signaling reporter system and investigated the expression of genes functioning downstream in the cytokinin response pathway. Results of these studies will also be presented. In the future we plan on isolating tissue along various growth axes in normal and *Hsf1* mutant leaf primordia using laser capture micro dissection and performing a transcriptome wide expression analysis. This will provide us with a global view of gene expression changes involved in hormone signaling control of maize leaf patterning.

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P89

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

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Auxin regulates almost every aspect of plant growth and development. A better understanding of the role of auxin is fundamentally important to basic plant biology and crop improvement. Previous research has demonstrated both conservation and diversification of the role of auxin in maize and Arabidopsis. This project will further our understanding of how auxin regulates shoot development, with an emphasis on maize shoot organogenesis.

To identify additional genes functioning in auxin-mediated organogenesis, we are characterizing 138 maize mutants with characteristic defects in vegetative and reproductive development. So far, we have mapped 44 mutants to 27 locations in maize genome. Fourteen of these loci had previously been cloned and an additional six genes have been cloned on this project. Currently, the genes underlying six loci are being identified by positional cloning and mapping populations are being constructed for the remaining 32 mutants. Many of these genes that have been cloned encode proteins required for auxin biosynthesis, transport and response. Preliminary phylogenetic analysis of eleven gene families has illustrated complex relationships amongst monocot and eudicot clades. Further phylogenetic characterization of all identified gene families in combination with comparative expression and synteny analyses, will test the conservation and diversification of the mechanisms of auxin action in all flowering plants.

www.AuxinEvoDevo.org

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P90

Auxin content, cell size and endoreduplication level in the mutant *defective endosperm*-18

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The maize mutant *de*18 maps to chromosome 10, bin 03, coincident with the umc1962 marker. The *de*18 accumulates substantially less dry matter in the endosperm than its normal counterpart. The auxin IAA levels in *de*18 endosperm are 15 times lower respect to the wildtype. The addition of synthetic auxins to the developing *de*18 grains rescues the wild type phenotype. The expression level of auxin regulated genes appears to be reduced in *de*18 during endosperm development, as revealed by microarray analysis. Auxin is involved in enhancing post-mitotic nuclear DNA synthesis (endoreduplication), that is positively correlated with cell enlargement and cell volume. We have investigated whether the reduced endosperm of *de*18 is due to impaired cell division and endoreduplication process, as a consequence of the low auxin levels. Nuclear endoreduplication level, number and size of cells have been measured in wild-type B37 and *de*18 kernels at 8, 12, 14 and 16 DAP with the optical miscroscope and computer image analysis, using the 3D model developed for maize endosperm. Observations of cells distribution with different ploidy levels in both genotypes, showed that at 8 DAP most of cells in the endosperm were 3C and 6C cells and they were restricted mainly to the outermost layers. Endoreduplication began in the nuclei of the central starchy endosperm cells (12C) and proceeded basally and outward until 16 DAP, where 96C and 192C nuclei were localized in the central part of endosperm. The most significant differences between *de*18 and B37 were detected at 8 DAP, where the mutant showed a deficiency in the ploidy level, number and volume of cells.

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P91

Bract suppression evolved in the grass family following a series of duplication events that created the *Tsh1* and *Ntt* gene lineages

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Vegetative growth in plants is dominated by leaf growth. After the transition to flowering, leaf growth is often significantly diminished, or even completely suppressed. Leaves that are present in the inflorescence are called bracts, and bract suppression has evolved independently in several angiosperm lineages including the grasses (Poaceae) and Brassicaceae. Genes regulating bract suppression have been identified in both maize and *Arabidopsis*. Interestingly, the genetic mechanisms controlling bract suppression in the grasses (maize) and Brassicaceae (*Arabidopsis*) are distinct. One gene important for bract suppression in the grass family, *Tsh1*, has a completely different function in *Arabidopsis*. In spite of their distinct expression patterns and functions, both *Tsh1* and its *Arabidopsis* ortholog *HANABA TARANU* (*HAN*) are capable of inhibiting lateral organ growth ectopically in *Arabidopsis*. In the grass family, *Tsh1* belongs to a clade that also contains two paralogs known as *Neck leaf1/Tsh1/Third outer glume-like1* and *2* (*Ntt1, 2*). A possible scenario for the evolution of bract suppression in the grasses involves gene duplications creating the *Tsh1/Ntt1/Ntt2* lineages, followed by neofunctionalization in the *Tsh1* lineage resulting in a novel bract suppression function in the grasses. We have isolated *Tsh1* and *Ntt* orthologs from early diverging grasses and grass outgroups to identify when the paralogous lineages arose. Our phylogenetic results indicate that the *Tsh1/Ntt* paralogs were created in a series of duplications. Furthermore, the *Tsh1* lineage was present already in *Joinvillea*, an outgroup to the grass family that does not suppress bract growth. This was surprising as it is unclear what the function would be of a *Tsh1* ortholog *Joinvillea*. We are currently investigating expression of the *Tsh1* and *Ntt* lineages in grasses and outgroups to see if changes in their expression domains correlate with a novel role in bract suppression .

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P92

Brassinosteroid control of sex determination in maize

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Brassinosteroids (BRs) are important regulators of plant growth and development. They share structural similarities with animal steroids, which are decisive factors of sex determination. We show that BRs control cell elongation and sex determination in maize revealed through characterization of the classical dwarf mutant *nana plant1* (*na1*), which also feminizes male flowers.

na1 carries a loss-of-function mutation in a key enzyme of the BR biosynthetic pathway. The mutant accumulates the predicted NA1 steroid substrate with a concomitant decrease of downstream BR metabolites. Treatment of wild-type maize with BR biosynthesis inhibitors completely mimicked both dwarf and tasselseed phenotypes of *na1*. Tissue-specific *na1* expression in developing anthers also indicates that BRs promote masculinity of the male inflorescence. Furthermore, we isolated multiple alleles of a second, non-allelic mutant, which shares key characteristics of the *na1* phenotype.

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P93

Characterization and Cloning of *tassels replace upper ear1* in Maize

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Selective elimination of male or female developmental processes in meristems affects the morphological distinctions between inflorescences in maize, as interplay of genes and hormones regulates plant architecture. The *tassels replace upper ears1* (*tru1*) gene of maize regulates plant architecture by restricting lateral meristem activities including shank elongation, and affecting subsequent floral meristem activities in inflorescence identity. By map-based cloning (total 4,729 mutant individuals) we isolated the *tru1* gene. *tru1* encodes a protein with a BTB/POZ domain and ankyrin repeats, highly similar to *BLADE-ON-PETIOLE1* (*BOP1*) of Arabidopsis. In Arabidopsis, *BOP1* has many functions, including in leaf morphogenesis and floral meristem identity. Additional mutant alleles of *tru1* were identified in a noncomplementation screen using EMS. Three mutant alleles (one insertion and two nonsense mutations) partially derepress branches on the main shoot. The top two or three axillary branches elongate into tassel-tipped branches and lower branches are progressively reduced, revealing a key role for *tru1* in regulating axillary branch growth. Detasseling experiments show that *tru1* functions downstream of a signal from the tassel that inhibits shank elongation. On the other hand, the *tb1*; *tru1* double mutant is phenotypically indistinguishable from the *tb1* single mutant, so mutation of *tb1* is epistatic to that of *tru1*. *tru1* mutants also have reduced internode length above the topmost axillary branch. In two conditions that affect these internodes, *tru1* mutation is epistatic: exogenous GA application, and altered auxin signal transduction in *bif2* mutants. Double-mutant analyses with *kn1*, *ts2*, *gt1* and *ba1* mutants are also underway to study potential interactions among these genes. *tru1* transcripts are detectable in root (DAP4-7), shoot (DAP7), embryo (DAP15), SAM (1-2mm, DAP30), tassel (3-5mm, 9-10mm), ear (2-7mm, 9-10mm), leaf (DAP21) during vegetative development and anthers during reproductive development. Future work will include detailed expression analysis of *tru1*, and morphological analysis of mutant plants, to elucidate *tru1* function in regulating inflorescence development in maize.

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P94

Characterization of *sterile tassel silky ear1*, a *PISTILATA*-like mutant in maize

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According to the classic ABC model of floral development the determination of angiosperm floral organ identity is controlled by the combinatorial activity of A, B, and C class genes. Although this model has proved accurate for many angiosperms (especially model eudicots), it is still unclear how much of it is conserved in the monocots. Mutants in monocot A,B, or C class genes can provide a valuable comparison to assess the conservation of this classic developmental model. In a screen for maize tassel floret mutants, performed previously, a novel mutant *sterile tassel silky ear1* (*sts1*) with homeotic conversions of floral whorls two and three was identified. The organs of whorl two (lodicules) and whorl three (stamens) in homozygous mutants were consistently converted to lemma/palea-like organs. This pattern of homeotic transformation suggested that the mutation could be a loss of function of a maize B class gene. Mapping and sequence data provided further support of this hypothesis, indicating that *sts1* is likely caused by mutation of the maize *PISTILATA* ortholog, *Zmm16*. To confirm that this is indeed the case, complementation crosses have been performed in order to generate *Zmm16*-YFP transgenic plants that are also homozygous for *sts1*. Data regarding the ability of the *Zmm16*-YFP transgene to rescue the mutant phenotype will be presented.

To further characterize the *sts1* phenotype, Scanning Electron Microscopy of a developmental series from wild type and *sts1* mutant floret meristems is underway. These SEM comparisons should provide further information on B class function in maize.

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P95

Characterization of a New *ROUGH SHEATH1* Mutant of Maize

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In maize, class 1 *knox* genes are expressed in the shoot apical meristem and encode transcription factors that are involved in cell fate decisions. A *knox*-like mutant was identified in a maize line that exhibits active *Mutator* transposition. This *knox*-like phenotype is evident as aberrant cell patterning at the leaf sheath/blade boundary and develops on more developmentally mature plants. SSR mapping indicates that the gene responsible for this mutant phenotype is closely linked to *rough sheath1* (*rs1*), a class 1 *knox* gene. In addition, a *Mu*-TIR/*rs1* PCR primer pair consistently amplified DNA in mutant plants. RT-PCR was used to qualitatively characterize the expression of eight class 1 *knox* genes in leaf tissues. *rs1*, *lg3*, *knox5*, *knox8* and *knox11* were all ectopically expressed in the mutant sheath/blade boundary; *rs1* was also ectopically expressed in mutant blade tissue. Preliminary histological analysis of this *Mu*-tagged *rs1* mutant indicates that there may be an expansion of sclerenchyma cells in sheath/blade boundary and that there is less lignin found in this region compared to wild-type plants. We also observed that these *Mu*-tagged *rs1* plants have abundant lesions on their leaf blades, which appears to be suppressed when plants also bear a *Mu*-tagged *WRKY1* allele. Current studies continue to investigate the histological and lignin content of plants segregating for *Mu*-tagged *rs1* and/or *Mu*-tagged *WKRY1* alleles.

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P96

Characterization of novel anther mutants in maize

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An early stage anther consists of three layers (L1, L2 and L3) that undergo regulated cell divisions to produce a mature anther with five distinct cell layers: the epidermis, endothecium, middle layer, tapetal layer, and meiocytes. The epidermis is solely derived from the L1 layer while the other four layers initiate from the L2. The L3 layer differentiates into the vascular bundle and connective tissue. Proper regulation of cell differentiation in the anther is crucial because the process is tightly connected with meiosis. Mutants with defects in this process cause male sterility. In order to further understand anther development in maize, male sterile mutants obtained from various public sources were analyzed. Allelism tests revealed 27 loci with pre-meiotic defects and cytological analyses allowed us to group the mutants into different classes depending on the nature of the phenotype. Among the 27 mutants, the phenotypes of *ms32, EMS63089, EMS71924*, and *ms-si-355* are presented. The former two have defects in layer differentiation; *ms32* shows additional divisions in the tapetal layer causing meiocytes to collapse and *EMS63089* has multiple defects in layer differentiation. *EMS71924* only exhibits two lobes compared to the four lobes in wild-type anthers suggesting an early defect in lobe separation. Among the four mutants, *ms-si-355* shows the earliest defects: anther structure is completely absent from the spikelet. Here, we will discuss the cytological results and cloning efforts for these mutants.

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P97

Characterization of the pollen-specific *stk1* and *stk2* genes in maize

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stk1 is the proximalmost gene in the *bz* gene island in 9S. Its predicted protein is highly similar to serine/threonine protein kinases, hence its name. A closely related gene, *stk2*, is found located in 4L. *stk1* and *stk2* are also found in sorghum and rice, and phylogenetic analysis shows that they are paralogous genes, in other word, the gene duplication happened before the maize-rice speciation event dated around 50 MYA (million years ago). Both paralogs are only highly expressed in pollen and in the mature tassel, but not in other tissues. We have obtained mutants of both genes: 4 *Ac* insertions for *stk1* and 3 *Mu* insertions for *stk2*. The *stk* genes function in pollen tube (PT) growth since mutations of *stk1* show reduced pollen transmission only when competing with wild type pollen. Mutations of *stk2* have a smaller pollen transmission effect, suggesting that *stk1* may play a more important role in pollen development. We have introgressed the *stk* mutations into a common genetic background and generated double mutants. The double mutant combination is essentially pollen lethal, as the double mutant pollen germinates at a very low rate *in vitro*, which results in rare recovery of the double mutant. We conclude that the *stk* paralogs play an essential, though slightly unequal, role in pollen development.

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P98

Clone and characterize the Tcb1 factor (s) that forms cross barrier between maize and teosinte

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Reproductive isolation blocks gene flow between species, maintaining species integrity and forming the basis for selection and evolution. However, the molecular and cellular mechanisms of how cross barriers form is poorly understood. Cross-pollination between maize and some strains of its ancestor Teosinte is unilateral, in that teosinte pollen is able to fertilize maize, while crosses in the opposite direction fail. This provides a model system to study the mechanisms of reproductive isolation. Previous research showed that a single locus, *teosinte crossing barrier1* (*tcb1*), can control this cross-incompatibility with the *Tcb1-s* haplotype conferring cross-incompatibility. The *Tcb1-s* haplotype consists of a pistil factor that rejects *tcb1* (maize-like) pollen and a pollen factor that allows *Tcb1-s* pollen to function on *Tcb1-s* pistils. Using map-based cloning, we are trying to identify and characterize the Tcb1 gene (s). Maize inbred line W22 sublines carrying *Tcb1-s* were established by reciprocal backcrosses of males carrying *Tcb1-s* of teosinte to *tcb1* W22 females. Out of a 15,000 mapping population, the pollen and pistil factors have been separated by recombination, and the pollen factor has been pinpointed to a region on chromosome 4 that spans 165 kb and contains several putative genes. To understand why crosses fail between maize pollen and Teosinte silks, *in vivo* pollen germination and pollen tube growth was tested after *tcb1* maize pollen was put on silks of maize plants carrying the *Tcb1-s* haplotype. Visualized by aniline blue staining, pollen tubes were found at the base of the maize silks 50 hours after pollination. However, in the Tcb1 silks, even though pollen tubes were observed, they only reached a distance of less than 0.5 cm on the top, which suggests that pollen germination is fine, but tube growth was blocked. Studies are underway to clone this gene and characterize its function.

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P99

Cloning and characterization of a dominant phyllotaxy mutant (Abphyl2) in maize

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Plant morphology and diversity are largely dependent on the establishment of phyllotaxy, which is initiated from a group of stem cells in the shoot apical meristem (SAM). Auxin is a crucial factor controlling phyllotactic patterns. Recently, studies on ABPH1 in maize have shown that cytokinin, as well as its crosstalk with auxin, play an important role in this process. abph1 mutations change maize phyllotaxy from alternate to decussate. In this study, a similar phyllotaxy mutant, Abphyl2 (Abph2) is described. Abph2 is dominant, and originated from a Chinese inbred line, and has a decussate leaf pattern that becomes visible at leaf ~ 4-5. Map-based cloning done in 3 genetic backgrounds has mapped Abph2 in a region of ~ 20kb on chromosome7, containing 5 predicted genes in the reference B73 genome. However, direct sequencing of the open reading frames of these 5 genes, as well as their transcript level analysis by normal RT-PCR and/or qRT-PCR, did not tell us which is the candidate gene. Next, a BAC library generated from the Abph2 mutant was screened using probes located within the 20 kb mapping interval, and identified 6 positive clones. One of them fully covers the 20 kb mapping interval. We are currently analyzing the sequence of this BAC to get clues into the molecular basis of the Abph2 phenotype. In addition, introgression lines were generated to monitor the influence of genetic backgrounds on Abph2 phenotype. Lastly, informed by the fact that PINFORMED1 (PIN1) maxima in the P0 is diminished in abph1, we are currently analyzing auxin distribution in Abph2 meristems, using PIN1-YFP and DR5-RFP as markers.

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P100

Colonization of roots of the Egyptian corn (Zea mays), Sesame (Sesamum indicum) and cotton (Gossypium barbadense) plants by the endophytic basidiomycete Piriformospora indica promotes their growth and salinity tolerance.

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The major threat to agricultural production in many areas in Egypt is salinity of soil or irrigation water. Contributing to efforts to solve this problem and directed by results from previous research [Verma, S. et al. (1998) Mycologia 90, 896-903] showing that the root-endophytic fungus, Piriformospora indica improves plant growth as well as resistance against root and leaf diseases and alleviate salt stress in many plants, pot experiments were conducted to assess effects of infestation by that fungus on growth performance of three of the important Egyptian crop plants and on their tolerance of salinity. In those experiments, tested plants were grown for 2 weeks in soil supplemented with 0, 100 and 300 mM NaCl solutions before being treated by presence or absence or of the fungus or its filtrate. Five weeks after inoculation, whole plants were harvested and divided into roots and shoots, and fresh weights of each were determined. The root and shoot biomasses were significantly enhanced due to infestation of plant roots by P indica and in some cases they were about twice of those of the controls. Although not as effective as the infestation by the fungus, application of fungal culture filtrate also promoted plant growth and biomass production. These findings show that P indica fungus could be exploited to increase salinity tolerance and to increase yield in Egyptian crop plants. They provide a guide to agriculturists to extend these tests to field experiments and to plan strategies to overcome salinity constraints against crop production.

Funding acknowledgement: Sohag University

P101

CsCl implicated a role for K^+ in the early growth of maize primary root

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Fresh weight of maize root and shoot above coleoptilar node decreased when 5 – 30 mM CsCl was applied. Elongation growth of the primary root also decreased within the same range of CsCl. The CsCl-inhibited root growth was partially restored, when 60 mM KCl was applied together. Analyses revealed that 10 – 30 mM CsCl competed with KCl, however, 5 mM CsCl did not, indicating that KCl acts in two different modes. Differential effects of CsCl depending on concentrations were observed also in radial expansion of subapical region of the primary root tip. Furthermore, CsCl above 10 mM induced *ZmKUP1* encoding K^+ transporter, in contrast that CsCl below 5 mM did not show any clear effect. Root apical meristem was not affected by CsCl treatment, indicating that the observed changes in the primary root growth was mainly due to alterations in cell expansion via complex mechanisms.

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P102

Deep sequencing of maize endosperm culture transcriptomes to understand the impact of the ROUGH ENDOSPERM3 splicing factor

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The maize *rough endosperm3* (*rgh3*) mutant encodes the U2AF35-related protein (ZmURP). In humans, URP is associated with the minor and major spliceosomes, but its biological role is unknown. Prior characterization of the *rgh3* mutant indicates that the gene is required to repress cell proliferation and promote cell differentiation. The *rgh3* mutants are highly proliferative in endosperm cell culture when compared to wild-type cells that undergo endoreduplication. Our data indicate that *rgh3* endosperm cells remain frozen in this early, undifferentiated stage throughout kernel development. Thus, Rgh3 is not a core splicing factor required for cell viability. We hypothesize that *rgh3* has an effect on RNA splicing and gene expression. To test this hypothesis, we compared *rgh3* and normal sibling transcriptomes in an RNA-seq experiment with the ABI SOLiD platform. About eight million reads from each genotype were aligned uniquely to the genome. These sequences mapped to 26% of predicted exons in the genome and detected >26,000 annotated genes. The *rgh3* mutation had subtle effects on transcript levels with only 87 genes showing a four-fold difference compared to wildtype. The expression of splice junctions was also compared in *rgh3* and WT samples. Future work will compare alternative splicing and gene expression in endosperm culture, roots, and aerial parts of young seedlings. Additionally, direct gene targets of Rgh3 will be identified using HITS-CLIP.

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P103

Development of the monocot sheathing leaf base: the role of NARROW SHEATH

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Leaves develop from the periphery of the shoot apical meristems (SAM) via the recruitment of leaf founder cells, a process that begins with the transport of auxin and subsequent downregulation of *knotted1* homeobox gene expression at the site of leaf initiation. Unlike eudicots in which the lateral recruitment of founder cells and correlated *knox* gene downregulation are localized to the site of leaf insertion at the node, monocot leaves are recruited from the entire circumference of the SAM, a process that eventually gives rise to the sheathing leaf bases observed in maize leaves. Mutations in the duplicate *wuschel*-like transcription factors *ns1* and *ns2* cause defects in leaf founder cell recruitment. ns mutant leaves are narrow and fail to form a sheathing base, a phenotype that correlates with failure to downregulate *knox* gene expression throughout the SAM periphery and failure to recruit founder cells for leaf lateral domains. Although the NS proteins do not traffic intercellularly, clonal analyses reveal that NS functions from two lateral foci to send a non cell-autonomous signal that recruits founder cells from the circumference of the SAM. Understanding the nature of this signal transduction pathway will elucidate the role of NS function during development of the sheathing leaf bases of monocot leaves. Transcriptomic analyses combined with cell-specific imaging of fluorescently-tagged cellular proteins indicate that NS function involves a complex interplay between cytokinin signalling and auxin. NS is required to localize the cytokinin 2-component signalling protein HP1 to two lateral foci within the SAM. The auxin response reporter DR5 reveals that lateral recruitment of leaf primordia from the SAM correlates with a progressive auxin response, a process that is prematurely halted in ns mutant primordia. Localization of the PIN1a auxin transporter suggests that lateral recruitment of maize founder cells correlates with the progressive transport of auxin from midrib to margin regions. The data suggest a model in which NS-mediated auxin and cytokinin signaling are required to form the sheathing bases of monocot leaves.

Funding acknowledgement: National Science Foundation (NSF)

P104

Duplicate *naked endosperm* genes encode ID domain transcription factors required for maize aleurone differentiation

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The outermost layer of the endosperm is a specific cell type called aleurone which is important for grain quality because of its high content in lipid and minerals compared to starchy endosperm. The aleurone layer is also an attractive system to study cell fate determination because of the plasticity of aleurone cell fate and its easy accessibility. Here we report the identification of *Naked endosperm (Nkd)* genes which are involved in aleurone differentiation in maize. The *nkd* mutant shows defects in aleurone cell identity and has approximately 3 outer cell layers instead of the single in wild type. However these outer cells do not contain dense granular cytoplasm typical of normal aleurone and have sporadic expression of a VP1-GUS transgene, which is a useful aleurone identity marker. The *nkd* mutant phenotype shows 15:1 segregation ratio in F2 populations suggesting two recessive genes are involved in this phenotype. We performed map-based cloning and found two homologous genes in syntenic regions on chromosomes 2 and 10 are tightly linked with this phenotype. The INDETERMINATE domain containing transcription factors IDDveg9 and IDD9 correspond to the *nkd1* and *nkd2* mutants on chromosome 2 and 10, respectively. An additional mutant allele for *nkd1*, which has a Ds transposon insertion in exon3, showed a *nkd* mutant phenotype when it was crossed with the *nkd2* mutant. The NKD proteins have nuclear localization signals and GFP fusion proteins showed nuclear localization. *Nkd* transcripts were detected in young leaves but are more highly expressed in developing kernels around 10 days after pollination. Further analysis of these gene and protein functions is ongoing.

Funding acknowledgement: United States Department of Agriculture (USDA)

P105

Effects of chromosome number variations on global gene expression

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Despite the widespread interest in aneuploidy, the molecular mechanisms that lead to phenotypic alterations in aneuploid organisms are still poorly understood. Moreover, it is not clear what gene interactions are involved in coping with gene dosage imbalance caused by aneuploidy on the global genomic level. Plants are more tolerant to aneuploidy than animals and present a good model for research. Here, we investigated aneuploidy effects on gene expression in the maize aneuploid that carried an extra copy of a small arm of chromosome 5 and exhibited several phenotypic traits, such as stunted growth, late development, partial tassel sterility, and knots in the leaves. We were primarily interested in understanding whether the phenotypic syndromes of aneuploidy could be attributed to a small number of affected genes or a complex network of gene interactions is involved. We also wanted to know whether different plant organs respond differently to aneuploidy. We compared expression levels of approximately 15,000 maize genes in meristems and leaves of aneuploid and wild type seedlings. In addition, we chose 30 genes that showed largest variation in seedlings and meristem-enriched tissues in response to aneuploidy and characterized their expression in eight different tissues and during early plant development in wild type and aneuploid plants. Our data demonstrated that different sets of genes are affected in different tissues and that many genes respond to aneuploidy by changing their expression pattern and becoming ectopically expressed. Such drastic changes of expression patterns are likely to contribute to phenotypic syndromes seen in aneuploid organisms.

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P106

Functional analysis of the Hairy Sheath Frayed1 mutation in maize

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Hairy SheathFrayed1 (Hsf1) is a semi-dominant, gain-of-function mutation that disrupts proximal-distal patterning in the maize leaf. The gene responsible for this phenotype has been cloned and is *Zea mays his-kinase protein1 (ZmHK1)* , one of the maize histidine kinase cytokinin receptors. Histidine kinase cytokinin receptors are part of a two-component signal transduction system that enables cells to respond to cytokinin and regulate development. The sensor histidine kinase receives the cytokinin signal at the cell membrane, autophosphorylates and initiates the transfer of phosphate to a series of downstream signaling proteins. Ultimately, the signal activates expression of a number of response regulator proteins which function to control many developmental programs. The binding of cytokinin to *ZmHK1* is thought to occur at the CHASE (cyclase/His kinase-associated sensing extracellular) domain. Sequence analysis of three *Hsf1* alleles, *Hsf1-1595*, *Hsf1-1603*, and *Hsf1-AEWL1*, revealed unique missense mutations in highly conserved residues of the CHASE domain. To better understand the impact these changes were having on *ZmHK1* function, we tested wild type *ZmHK1* and the three *Hsf1* alleles in an *E coli* his-kinase beta-galactosidase assay system. Each of the *Hsf1* mutant alleles shows constitutive cytokinin signaling activity in the absence of cytokinin and increased response to cytokinins compared to wildtype *ZmHK1*. Constitutive cytokinin signaling activity of *ZmHK1* in the *Hsf1* mutants causes upregulation of at least one of the downstream response regulators. How this leads to altered proximal-distal leaf patterning is not yet clear but we are actively investigating this question.

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P107

Genetic Control of Natural Variation in Maize Meristem Architecture

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The shoot apical meristem (SAM) is an undifferentiated group of stem cells that initiate all shoot-derived organs. The SAM generates lateral organs progressively as the plant grows, while simultaneously preserving enough meristematic stem cells to retain its function. As a result, the overarching structure of the meristem remains relatively constant during vegetative development. Here, the genetic control of SAM architecture and seedling leaf phenotypes, the parent-of-origin effect on SAM architecture, and the range of natural variation in maize SAM architecture was examined. To map loci controlling SAM architecture and seedling leaf phenotypes the intermated B73 x Mo17 recombinant inbred line (IBM) population was examined. SAM height, width, and arc length from the P1 cleft were measured in longitudinal histological median sections from 14-day-old seedlings. Quantitative trait loci (QTL) analysis identified six QTLs that control natural variation in maize SAM architecture. Seedling leaf traits (length, width, height) were also measured in the IBM RIL population and 21 QTL were identified, two of which were found to be potentially in common with SAM architecture QTL. Validation of large-effect and coincidental QTL was performed using introgression lines of B73 and Mo17. F1 progeny derived from reciprocal crosses of B73 and Mo17 showed that SAM height and arc length exhibited mid parent values. To examine the extent of natural variation in maize for SAM architecture, the nested association mapping (NAM) parental lines were investigated, where it was found that B73 and Mo17 capture a majority of the natural variation for height and arc length identified in the NAM parents.

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P108

Genetic and transgenic analysis of Brassinosteroids functions in maize

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Brassinosteroids (BRs) are a type of phytohormone that has important roles in plant development such as sex determination, internode elongation, and leaf development. Even though they are well studied in model species, their role in maize development is poorly understood. To address this issue, a transgenic approach was pursued. Four genes related to the either BR biosynthesis or signaling were manipulated by overexpression or RNAi suppression to investigate their roles in the growth and development of maize. We generated overexpression transgenic plants for *DWF4, BIN2*, and *BES1*, and knock down transgenic plants for *DWF4, BIN2*, and *BRI1* genes. The preliminary results suggest that similar to other plants, BRs also regulate plant height in maize. In addition, they appear to have novel functions in maize, influencing reproductive development and leaf morphology. Some of dwf4RNAi events produced plants showing a tasselseed phenotype, where female florets and kernels develop on the tassel. Additionally, several showed a terminal ear phenotype in which an ear formed one node below or directly below the tassel. 13 out of 17 bri1RNAi transgenic events produced dwarf plants with dark green, upright, and twisted leaves. Plants obtained from the bin2RNAi events showed variable phenotypes. Some plants showed unusual leaf morphology and decreased stature with shortening of the upper internodes. Leaves were unusually long and showed crenulated leaf margins. These plants were sterile. Transgenic BES1 overexpression events produced dwarf as well as tall plants. The dwarf plants produced ear shoots on lower nodes near the soil surface.
Genetic studies have also identified a semi-dwarf mutant that has upright, dark green and twisted leaves, which are phenotypes of BRs mutants in other species, and which resemble the BR knockdown phenotypes above. This mutant maps to a small interval containing a homolog of BAK1, involved in BR signaling in other species.

Funding acknowledgement: Plant Sciences Institute, Iowa State University

P109

Genome-wide analysis of KNOTTED1 binding sites using ChIP-seq

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KNOTTED1 (KN1) and other class I homeobox (KNOX) transcription factors are involved in the establishment and maintenance of plant meristems. According to the current model, KNOX proteins act in part through the modulation of hormones such as cytokinin and gibberellin, and through the negative regulation of secondary cell wall biosynthesis. So far, direct KNOX targets have remained elusive, in part because of the degenerate nature of KNOX consensus binding sites. To identify the genes regulated by KN1 on a genome-wide level, we used KN1-specific antibodies to perform chromatin immunoprecipitation (ChIP) followed by high-throughput sequencing of ChIP DNA (ChIP-seq). We identified thousands of loci occupied by KN1 in immature ears, many of which are located in the vicinity of annotated genes and a significant proportion is specifically located within introns or UTRs. Manual validation by quantitative ChIP-PCR confirmed the binding of KN1 to a subset of loci in immature ears as well as in immature tassels and the shoot apical meristem of wild-type but not kn1 null mutant, supporting the validity of the dataset. Among the putative KN1 targets, we found the most significant enrichment for genes involved in hormone metabolism and signaling, revealing that the main mechanism by which KN1 controls development is by interfering with all the major hormone pathways.

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P110

Identification of pan2 as a leucine-rich repeat receptor-like kinase promoting division asymmetry via a proteomic strategy

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Stomata in maize are composed of a pair of guard cells flanked by a pair of subsidiary cells that regulate stomatal aperture. Subsidiary cells form by means of asymmetric divisions of subsidiary mother cells (SMCs). SMC divisions are oriented so that the subsidiaries always form adjacent to guard mother cells (GMCs). We are studying SMC divisions as a model for asymmetric division in plants. Two *pangloss* genes (*pan1* and *pan2*) promote the polarization of SMCs prior to their asymmetric division. Previous work demonstrated that *pan1* encodes a leucine-rich repeat receptor-like kinase (LRR-RLK), which accumulates in SMCs at the site of contact with GMCs in a pan2-dependent manner, suggesting that it might function as a receptor for a polarizing cue from the adjacent GMC (1). Using a proteomic approach, we have now identified the *pan2* gene. Membrane fractions from wild type plants and *pan2* mutants were isolated from leaves at the stage where stomatal divisions occur, labeled with isobaric tags (iTRAQ reagents), mixed, and analyzed via LC-MS/MS. Peptides of an LRR-RLK encoded by a gene near *pan2* were depleted in *pan2* mutants. Sequencing of this gene in plants carrying 4 independent *pan2* mutant alleles confirmed that this is the *pan2* gene. Experiments are now underway to investigate the localization of this LRR-RLK, its interaction with pan1, and its in vitro kinase activity.

1. Cartwright, H.N., Humphries, J.A. and Smith, L.G. (2009). PAN1: A receptor-like protein that promotes polarization of an asymmetric division in maize. *Science* **323**:649-651.

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P111

Identification of proteins that interact with the maize transcription factor KNOTTED1

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A critical component to plant development is the establishment and continual replenishment of meristems, a group of totipotent cells, from which all plant organs arise. Class 1 *knotted1*-like homeobox (*knox*) genes are involved in the maintenance of meristems. It is known that *knox* genes play a major role in plant development, however, the specific details behind the mechanisms involved are lacking. *Knox* genes encode transcription factors containing the three amino acid loop extension (TALE) family homeodomains. Animal studies have demonstrated that TALE transcription factors dimerize with other TALE and non-TALE transcription factors. Dimerization is necessary for nuclear import, protein stability, and nucleotide binding affinity. Previous work has identified *knotted1* interacting protein (KIP) through a yeast two-hybrid screen. KIP is a TALE transcription factor belonging to the BLH (*bel1*-like homeobox) subfamily that was shown to dimerize with KN1 and bind DNA with high affinity. KNOX proteins in other species have also been found to interact with BLH transcription factors as well as proteins involved in inter- and intra-cellular protein trafficking. Until now, KIP was the only known KN1 interacting protein among the *Zea mays* TALE family. To determine whether KN1 also interact with other BLHs, we cloned all the *blh* genes in *Zea mays*. Using yeast two hybrid assays, we found that most of them interact with KN1. Furthermore, proteins transiently expressed in tobacco leaves were pulled down with KN1. We are continuing to seek KN1 interactors by mass spectrometry of KN1 complexes isolated from meristem nuclei extracts.

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P112

Inbred-specific modifiers of the maize ta-siRNA pathway

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The ta-siRNA pathway plays an important role in the establishment and regulation of dorsiventral (abaxial/adaxial) leaf patterning in maize. Several mutants in the ta-siRNA biogenesis pathway disrupt this process and exhibit defects in leaf polarity, which are characterized by sectors of abaxial identity on the adaxial leaf surface and/or by radialization of the leaves. The severity of these phenotypes is largely dependent on the inbred background, with specific inbreds e.g. A619, Mo17 and W22, typically enhancing ta-siRNA biogenesis mutants, whereas other backgrounds e.g. B73, lead to a weaker phenotype. This inherent diversity across different maize inbreds provides a useful tool to discover novel enhancers/suppressors of small RNA biogenesis or function, and/or inbred-specific targets of the ta-siRNA pathway. We are utilizing the *leafbladeless1 ragged1 (lbl1-rgd1)* allele, an ortholog of *AtSGS3 (SUPPRESSOR-OF-GENE-SILENCING3)*, and *dicer-like 4 (dcl4)* mutants, both important components of ta-siRNA biogenesis, to identify potential modifiers. Currently we are using mapping populations to fine map inbred-specific enhancers/suppressors. In addition, we are performing a transcriptome analysis of the *lbl1-rgd1* mutant embryos and non-mutant siblings in inbred backgrounds that condition either a moderate or a severe embryo lethal phenotype. By combing these datasets we seek to achieve a better resolution of the factors contributing to inbred-specific differences with respect to the ta-siRNA pathway.

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P113

Influence of sucrose on in vitro callusogenesis of Lancaster inbred lines

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Lancaster germplasm is an important group of maize genotypes which is widely spread in initial and elite commercial breeding material in Ukraine. It contains gene complexes providing drought tolerance, high productivity, determined duration of vegetation. The optimization of in vitro cultivation is necessary step for further genetic modification and somaclonal programs of Lancaster genotypes.
The effect of sucrose on callusogenesis was investigated for three inbreds of commercial germplasm Lancaster: DK420-1 (subgermplasm Oh43), DK298 (subgermplasm Mo17/Oh43), DK633 (subgermplasm Mo17). Immature zygotic embryos, 1-1.5 mm in length, were harvested in 10-12 days after self-pollination from field donor plants and cultivated on N6 medium modified with 100 mg/l myo-inositol, 100 mg/l casein hydrolysate, 10 mg/l silver nitrate, 690 mg/l L-proline, 1 mg/l 2,4-dichlorophenoxyacetic acid, 0,1 mg/l abscisic acid and two levels of sucrose concentration – 30 g/l and 60 g/l.
Through 30 days in culture immature embryos of DK420-1 and DK298 produced different types of calli, but DK633 revealed only swollen scutellums. It was established that the increase of sucrose concentration in induction medium in twice had provoked the increase of frequency of total callusogenesis, frequency of morphogenic callus initiation and frequency of callus type I initiation. The degrees of all effects depended on genotype. For inbred DK420-1 under 60 g/l of sucrose the optimization of callus type I induction was observed. For DK298 under 30 g/l of sucrose the predominant production of calli type II, but under 60 g/l of sucrose - calli type I was received. The amount of inbred DK633 embryos with swollen scutellum was higher under increased sucrose content. Thus, sucrose concentration in induction callus medium can be an active mechanism of regulation of frequency and type of callusogenesis.

Funding acknowledgement:

P114

Investigation of molecular mechanisms controlling determinacy of the spikelet-pair meristem

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The characterization of molecular mechanisms controlling branching of cereal inflorescences, and the identification of novel genes that participate in this process are crucial for the improvement of yield traits of commercial crops. In current research we use maize mutants, ramosa1 (ra1), ramosa2 (ra2) and ramosa3(ra3), which have increased branching in the inflorescences, in order to characterize molecular networks regulated by these genes. First, we used ILLUMINA transcriptome analysis for identification of the networks regulated by the RAMOSA gene activities. Our preliminary analysis presents comparison of the transcription dynamics of the several transcription factors and response regulators gene families in the wild type and ramosa mutants. Second, we have analyzed differences in the expression patterns of the auxin response marker DR5:: mRFP.ER in wild type and ramosa mutant inflorescences. Here we detected expansion of the DR5 signal in the developing short branch meristems of ramosa mutants. Third we have performed ramosa3 enhancer / suppressor screens, where 63 putative modifiers were identified. In parallel, we present development of new maize fluorescent marker lines: pZmWUS1:: mRFP.NLS, marking central meristem zone and pRA3::mRFP.NLS, marking the RAMOSA3 expression domain. In order to gain a better understanding of the developmental dynamics between wild type and the ramosa mutants, these markers are being introduced into the mutant backgrounds. Combination of the Illumina transcriptome analysis with enhancer/suppressor screens and imaging starts to uncover the major networks participating in the regulation of the maize inflorescence branching.

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P115

Isolation of defective kernel mutants from an open-pollinated EMS population to identify maternal effects on seed developmental processes in maize

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The development of the maize seed is affected by the balance of paternal and maternal genomes. In a wild-type seed the diploid embryo contains an equal number of maternal and paternal genomes (1:1). The triploid endosperm, however, contains two maternal and one paternal genome (2:1). Variation from this 2:1 balance leads to defective or failed seeds. To better understand the genes responsible for this balance we have initiated a screen for maternal-effect seed lethality mutants. Maternal-effect mutations could be caused by dosage-sensitive/haploinsufficient regulators of seed development, gametophytic factors, or imprinted genes. 5280 EMS-treated families of B73 and W22 from the Maize Tilling Project were open pollinated and screened for defective kernels (dek). At least two ears were shucked in every family and dek were observed, the rest of the family was shucked. Lines with multiple dek-containing ears were scored for normal, collapsed, and loose-pericarp seeds. A fully penetrant maternal-effect mutation would cause a 1:1 ratio of normal to dek. Any ears with more than 25% dek could potentially carry a maternal-effect mutation with less than full penetrance. Families with less than a 3:1 ratio of dek , could contain a recessive dek allele and were excluded from further analysis. Out of 17,000 individual plants, 94 families passed the initial screen. Of these, nine ears from six families showed a ratio that was not different from 1:1 by chi-squared testing. Three families had two ears with a 1:1 ratio. An additional twenty ears had more than 25% dek kernels and rejected the chi-squared test for 3:1 segregation. These families may have mutations in biological pathways that must be present in the maternal lineage for seed development to progress.

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P116

Live-cell imaging of ZmRAB2A1-YFP and ZmRAB1A-CFP with Golgi and ER during maize leaf cell expansion show both overlapping and independent patterns of expression

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Maize leaves initiate at the base of the shoot apical meristem and undergo progressive cell division followed by cell expansion and differentiation. Proper cell expansion requires cell wall biosynthesis, which involves the synthesis of cellulose microfibrils at the plasma membrane and the synthesis, sorting, and secretion of wall matrix components. We are interested in understanding how these processes are coordinated and compartmentalized during leaf development. Genetic approaches have shown that small GTPases in the RAB protein family participate in vesicle sorting during leaf cell expansion in maize. Among the large RAB family, RAB1 and RAB2 in nearly all eukaryotes function in vesicle trafficking related to the nuclear-associated ER and Golgi subcellular compartments. On the other hand, ZmRAB1 and ZmRAB2 have both duplicated further in maize and each sub-group appears to have acquired developmental and cell-specific functions. To understand when and where ZmRAB1 and ZmRAB2 function in maize, we have tagged each protein with fluorescent markers and studied their *in vivo* localization during cell growth in relation to each other and to markers of the Golgi and ER compartments.

ZmRAB2A1-YFP partially co-localizes with the ER marker, GLOSSY8-mRFP, in doubly fluorescent stable transgenic lines. Transient expression of a Golgi marker construct, XYLOSYLTRANSFERASE-mRFP, in ZmRAB2A1-YFP maize leaf cells shows similar co-localization and extensive Golgi distribution throughout the cytoplasm of young, expanding cells. Live-cell imaging suggests comparable movement of Golgi-associated ZmRAB2A1-YFP and overlap between ZmRAB2A1-YFP and ZmRAB1A-CFP with directed movement toward the cell periphery. ZmRAB2A1 and ZmRAB1A1 must also function independently because protein expression of ZmRAB2A1-YFP and ZmRAB1A-CFP along the leaf gradient show both overlapping and independent domains. Bioinformatics approaches are being used to seek potential interacting proteins to be used for further localization and functional studies.

Funding acknowledgement: National Science Foundation (NSF), Department of Energy (DOE)

P117

Maize *csmd1* exhibits pre-meiotic somatic and post-meiotic microspore and somatic defects but sustains anther growth

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Maize male reproductive development is complex and lengthy, and anther formation and pollen maturation are precisely and spatiotemporally regulated. Here, we document that *callose, somatic and microspore defect 1* (*csmd1*), a new male sterile mutant, has both pre-meiotic somatic and post-meiotic gametophyte and somatic defects. Nuclear staining, bright field microscope, confocal microscopy, and Volocity software charted chromosome behavior, cell developmental events, and cell dimensions. Aniline blue staining and quantitative assays were performed to analyze callose deposition, and expression of three callose synthase genes was measured by qRT-PCR. Despite numerous defects and unlike other maize male-sterile mutants that show growth arrest coincident with locular defects, *csmd1* anther elongation is nearly normal. Pre-meiotically and during prophase I, there is excess callose surrounding the meiocytes. Post-meiotically *csmd1* epidermal cells have impaired elongation but excess longitudinal divisions, and uninucleate microspores cease growth; the microspore nucleoli degrade followed by cytoplasmic vacuolization and haploid cell collapse. The single vascular bundle within *csmd1* anthers senesces precociously, coordinate with microspore death. Although *csmd1* anther locules contain only epidermal and endothecial cells at maturity, locules are oval rather than collapsed, indicating that these two cell types suffice to maintain an open channel within each locule. Our data indicate that *csmd1* encodes a crucial factor important for normal anther development in both somatic and haploid cells, that excess callose deposition does not cause meiotic arrest, and that developing pollen is not required for continued maize anther growth.

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P118

Maize DH production - In search of effective colchicine-alternatives

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Doubled-haploid (DH) maize lines, in general, are produced using colchicine to double chromosome sets of haploid seedlings. However, some disadvantages in using colchicine are its high toxicity to mammals as well as its efficiency in doubling chromosome sets. Therefore, research presented here is aimed at identifying colchicine-alternatives for maize DH production.

In a first step, a screening system was developed for evaluating physical and chemical treatments to substitute colchicine. Root tips of maize seedlings (2n) were treated with APM (amiprophos methyl), oryzalin, or pronamide. An increased amount of cells containing doubled chromosome sets (4n) were detected. In the next step the efficiency of doubling chromosome sets was evaluated by treating haploid maize seedlings. Furthermore, conditions of treatment with colchicine were optimized e.g. the time point for initiating the treatment. Compared to colchicine, treating haploid seedlings with APM, oryzalin, or pronamide high doubling rates were not achieved. Furthermore, conditions of treatment prior to applying a mitotic inhibitor did not influence the doubling rates.

We embarked on a high-throughput screening system for detecting effective colchicine-alternatives. The aim was to identify those chemical compounds that might have an effect on the formation of the spindle apparatus. For technical reasons we could not use maize seedlings. Thus, a transgenic line of *Arabidopsis thaliana* L. with a fluorescent marker (GFP) for detecting depolymerization of microtubules was employed. In a first screen the presence or absence of a GFP signal was used as indicator for blockage of mitosis. This screen yielded 78 compounds which quenched fluorescence. In a second more detailed screen those compounds are being evaluated for their capability of doubling chromosome sets. Afterwards, putative inhibitors of mitosis will be tested on haploid maize seedlings.

Funding acknowledgement: Fiat Panis Foundation

P119

New Dominant Mutants From EMS Mutagenesis

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As a result of treating corn pollen with EMS to help colleagues find significant new mutants in their specific areas of emphasis, I have been able to view large M1 plantings and recognize new dominant mutant types, both whole plant cases and half plant chimeras. Many of these mutants were saved by selfing or by outcrossing to normal plants and observing the progeny. From over 300 putative cases noted, 251 were validated and subjected to tests to confirm, evaluate and characterize them as mutants. Of these, 84 proved to be good, clear and viable cases, which could be maintained as stocks and had relevant data and clear photographic images. This group is of special interest because dominant mutants are quite rare from EMS mutagenesis (200 times more rare than recessives) and thus are more likely to be something not previously observed. Seed samples and relevant data for each of these mutants have been sent to the Maize Genetics Stock Center, and similar data along with high-resolution photo images are available at MaizeGDB. The purpose of this poster is to call attention to these mutants, which are freely available to colleagues and students, and hopefully will lead to their characterization and location in the maize genome using some of the exciting new technologies now available.

The details of treatment, problems, consequences of handling, and theoretical considerations are found in an earlier publication: Neuffer, et al; 2009. All our images, posted at MaizeGDB, are of high resolution and can usually be digitally enlarged to reveal often striking details about each mutant. These mutants are a unique and valuable resource, but none have been definitely placed in the maize genome. We encourage colleagues and students to join us in doing so.

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P120

New maize mutants defective in female gametophyte development

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In plants, the diploid sporophytic phase ends in meiosis to initiate the haploid gametophytic phase. Despite their reduced size the egg-producing structure, the embryo sac and sperm producing structure, pollen grain are genetically active and phenotype reflects the haploid allele type. Gametophytes require basic cell processes, as well as specialized reproductive processes like pollen-embryo sac recognition and fusion. The genetic basis for gametophyte function and development is poorly understood, primarily due to reduced recovery of deleterious mutations. Mutations in female gametophyte specific genes result in characteristic reduced fertility and seed set phenotypes. Using Robertson's *Mutator (Mu)* transposon mutagenesis, we have identified a collection of maize (*Zea mays*) female gametophyte mutants in a screen for semi-sterility. The *indeterminate gametophyte 2 (ig2), indeterminate gametophyte 3 (ig3), embryo sac defective1(esd1) and small pollen1(sp1)* mutants exhibit a range of embryo sac abnormalities, including extra polar nuclei, absence of antipodal cell clusters and reduced embryo sac size. We are performing detailed analysis of the cytological defects caused by these mutations and are using map based cloning approaches, coupled with high-throughput sequencing of DNA flanking *Mu* insertion sites to identify the genetic lesions in these mutants. We predict these mutants will provide novel insights into the biology of female gametophyte development in plants.

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P121

Non-additive protein accumulation patterns in maize hybrids during embryo development

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Heterosis describes the superior performance of heterozygous F_1-hybrid plants compared to their homozygous parental inbred lines. In this study, heterosis was surveyed in embryos of the reciprocal maize F_1-hybrids UH005xUH250 and UH250xUH005, 25 and 35 days after pollination. First, length, weight, and the time point of seminal root primordia initiation were determined in the hybrids and their parental inbred lines. Subsequently, a proteome analysis based on two-dimensional gel electrophoresis (2-DE) of soluble proteins of the reciprocal hybrids and their parental inbred lines revealed that 141 of 597 detected proteins (24%) exhibited non-additive accumulation in at least one hybrid. Electrospray ionization-tandem mass spectrometry (ESI-MS/MS) analyses and subsequent functional classification of the 141 proteins demonstrated that development, protein metabolism, redox-regulation, glycolysis, and amino acid metabolism were the most prominent functional classes among non-additively accumulated proteins. In 35-day-old embryos of the hybrid UH250xUH005, a significant up-regulation of enzymes related to glucose metabolism which often exceeded the best parent values was observed. A comparison of non-additive protein accumulation between rice and maize embryo data sets revealed a significant overlap of non-additively accumulated proteins suggesting conserved organ- or tissue-specific regulatory mechanisms in monocots related to heterosis.

P122

Observations of an Ear – Heterosis in Potential kernel Number of a Hybrid Derived by the Mating of Sister Lines

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Maize inbred lines CG60 and CG108 are Iodent sister-lines, which share ~70% of their genome through co-ancestry. Despite this relatively high degree of co-ancestry, the F1 between these 2 lines exhibit heterosis for grain yield (38% above the mid-parent). This percentage of heterosis for grain yield is modest in comparison to the 50-65% typically observed in commercial maize hybrids, but relatively high when considering the genetic similarity of the parents. Equally intriguing is that the heterosis for grain yield of CG60xCG108 is not due to differences in the number of rows of kernels, but due to changes in the number of kernels per row. Observations from an ear initial developmental time experiment, suggest that CG60xCG108's increase in kernels per row is not due to an increase in the duration of development of the female inflorescence; but is due to an increase in the rate of reiteration of lateral meristems produced by the inflorescence meristem, and the fate of the lateral meristems produced late in the developmental period of the female inflorescence. The later lateral meristems develop into florets that have a "tassel-like" appearance and presumably do not count towards potential kernel number. The proportion of "tassel-like" florets to total florets, is greater on the inbred lines' ears than that on the ears of the hybrid. The observations made have provided developmental time points of interest: 1) V9-V10 is the mid-section of the steep, linear portion of the developing female inflorescence's growth curve; 2) V13-V14 is the ending of the female inflorescence meristem's period of lateral meristem production. With the intent of identifying genes and allelic interactions that explain the heterosis in kernel number per row, future experimentation will involve differential gene expression analysis of data generated from sequencing the transcriptome of tissue taken from the developing female inflorescences at the time points of interest.

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P123

Pattern of vegetative architectural development in green millet (Setaria viridis) under varied light regimes

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Vegetative architecture refers to the three-dimensional arrangement of branches in space. In grasses, vegetative architecture influences the ability of the plant to compete for light and other resources and ultimately affects economically important traits such as grain yield and biomass. Domesticated grasses generally exhibit reduced branching compared to their wild ancestors, especially in the subfamily Panicoideae, as seen in comparisons between maize and teosinte, sorghum and its wild relatives, and foxtail and green millet. Green millet (Setaria viridis) is an especially attractive model for branching due to its small size and complex branching patterns, and because of the recent genome sequencing of both green millet and its domesticated relative foxtail millet (Setaria italica). Patterns of vegetative development in green millet were analyzed in non-limiting nutrient and water regimes and under two different planting densities to determine the effect of light regime on vegetative architecture. In general, four orders of branches were observed, with the pattern of development on secondary and tertiary branches recapitulating the pattern of the culm. This pattern was consistently observed under reduced planting density, however, at high planting density, size and number of axillary branches was highly variable., with individual plants exhibiting very different growth patterns. The range of variation seen provides insight into developmental growth potential in this species and in other panicoid grasses.

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P124

Patterns in Lesions and Leaves

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The disease lesion mimic mutants produce macroscopic lesions on the surfaces of leaves. Certain mutants can exhibit a ``bull's eye'' pattern of lesions: a set of concentric zones that alternate between lesioned and transitional tissue. We have observed these patterns in a winter greenhouse with daily temperature fluctuations (*Les1*), and in the field during the summer (*Les1, Les3, Les6*, and *Les12*).

While the molecular mechanisms of lesion formation are unclear, the spatial patterning of the bull's eye indicates that lesion formation can oscillate: either the production of a recruitment signal that diffuses radially from the initially switched cell to its neighbors, or its reception by each annulus of cells, repeatedly stops and restarts. We have observed clusters of lesions formed by one-half, one, and two or more cycles of oscillations. The signal is eventually attenuated: lesions are fewer, smaller, and more widely spaced as their distance from the center increases, suggesting a threshold concentration of signal is needed. Elliptical zones are observed more often than circular ones, consistent with known gradients of cell expansion and elongation in the leaf. The oscillation --- a temporal patterning --- can be qualitatively explained by a simple model that assumes lesions initiate randomly.

Lesion patterns may reflect the spatial arrangement of leaf cells as well as stochastic processes. Close-up photography of the adaxial leaf surface of Mo20W, W23, and M14 shows differences in the architectural patterning of cell types. We suggest that the distribution of lesions on the leaf could depend on interior reflection of incident light at particular types of cell junctions; and that signal diffusion through the tissue could exploit certain arrangements of cell-to-cell contacts. Such effects could explain some of the phenotypic differences of the mutants among these lines.
Better characterization of the lesion and leaf patterns is needed.

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P125

Phase change in normal and Cg1-overexpressing Brachypodium

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Phase change, the transition from juvenile to adult, is required for the eventual flowering of plants. This process can also involve a shift in patterns of differentiation of vegetative organs, especially leaves. Phase-specific characters in maize include differences in epidermal wax, trichomes, shape of epidermal cells in cross and paradermal section, and extent of lignin contribution to cell walls. We examined Brachypodium, a small, fast-cycling grass that is easily transformed to see whether similar patterns indicative of vegetative phase change were present. We found that this pooid grass, which flowers after nine leaves in 20-hour days (and after 16 leaves in 12-hour days), shows anatomical variation consistent with phase change starting during the differentiation of leaf 5, but after differentiation of leaf 4 is complete, in both 12 and 20-hour days. The first four leaves lack lignin in cell walls and have straight walls in pavement cells. Leaf five and later-formed leaves have an increasing proportion of epidermal cell walls that are composed of lignin and show crenulation, characters associated with the adult phase in maize. Adult-type cells first appeared at the margins and flanking the midrib, and in later-formed leaves, also at the base of the blade. Interestingly, no leaf was composed entirely of adult-type cells. Leaf length was also associated with phase change: tillers on this highly-tillered species showed a progressive increase in proportion of adult morphology. Overexpression of the maize gene Corngrass1, which in maize conditions a prolonged juvenile phase, resulted in a delay both in flowering (17 leaves, in inductive photoperiod) and in the appearance of adult anatomical traits (at leaf 13). Thus, although the number of leaves before flowering triggered by either lack of inductive photoperiod or Cg1 overexpression is similar, the timing of vegetative phase change is not.

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P126

Phase-Specific Gene Expression Patterns in Maize Leaves

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Vegetative phase change is the developmental transition in higher plants from a juvenile to an adult program of vegetative growth which confers floral competence. In maize, the first 4-5 leaves are juvenile, with a waxy, glabrous epidermis, followed by 2-3 transition leaves, which are a mosaic of juvenile and adult tissues, 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 likely involve hundreds of genes. Previous microarray studies (Strable et al., 2008) identified several hundred maize genes as upregulated in either juvenile or adult leaf primordia. We are measuring the expression of selected downstream genes throughout the early stages of development in the first 10 leaves by RT-PCR. We aim to use these expression profiles to identify genes that have strongly correlated expression and then to seek elements of a shared regulatory system. Photosynthetic genes make up the largest class of juvenile-upregulated genes from our pilot microarray study and thus are an initial target of study. We have also profiled representative members of the transcription factor families which are the immediate targets of MiR156/172. Significant correlations in expression have been found between genes, as well as inter- and intraleaf variations in the expression of individual genes.

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P127

Phenotypic Characterization and Mapping of a *ragged leaf2* mutant of Zea mays

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Leaves emerge from the flanks of the shoot apical meristem, are asymmetrical along the adaxial/abaxial plane from inception and have tissues with distinctive features. We have characterized a novel mutation, *ragged leaf2*, and made efforts toward cloning the gene. *ragged leaf2* is a recessive mutant that arose in an EMS-mutagenized population of inbred A619 and was roughly mapped by Sequenom analysis to bin 2.07; fine-mapping is ongoing. Typical manifestations of the phenotype observed by SEM and several types of light microscopy are striate sectors of colorless tissue present in the leaf blade that form all types of trichomes on both abaxial and adaxial surfaces. Mutant sectors are a mosaic of cell types. Often the sectors will tear and make the plant look ragged or slashed. We have observed that the phenotype usually appears beginning in leaf 3, may vary in penetrance and expressivity and be affected by environment. Potential candidate genes will be discussed.

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

P128

Pollen Germination in Maize Does Not Require Changes in the Transcriptome

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One objective for our group is to better understand the molecular and genetic basis of pollen and pollen tube function, given its critical role in seed production and its importance as a means of gene flow between plant populations. We compared gene expression levels between seedlings, mature pollen and *in vitro*-germinated pollen from two different inbred lines (B73 and W22) using the Arizona microarray (www.maizearray.org). As expected, a large number of probes (>10,000) showed significant expression differences between pollen and seedling. In addition, a substantial number of probes (>1000) detected significant differences between the two inbred lines in either seedling or pollen. In contrast, no probes detected significant quantitative differences between RNA species in mature and germinated pollen. Furthermore, based on Gene Ontology term enrichment analysis, transcripts in mature pollen are enriched in several categories associated with active metabolism. This contrasts with the transcriptional profile of mature pollen in Arabidopsis, which is not enriched in metabolic processes, and shows significant change upon germination of the pollen tube (Wang et al. 2008). This suggests that, in contrast to Arabidopsis, mature pollen in maize (which remains 'partially hydrated' at anthesis) is metabolically active and does not rely upon novel patterns of transcription for germination. Experiments using the transcriptional inhibitor actinomycin D support this hypothesis, as maize pollen germination is relatively insensitive to the drug. Furthermore, comparative genomic analysis of the transcripts of mature pollen in maize and of post-germination pollen in Arabidopsis shows a significant overlap in orthologous genes. We are using new sequence-indexed mutant resources in maize (UniformMu and Ds Mutagenesis) to test whether this set of orthologous genes is associated with important functions in male gametophyte development.

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P129

Positional Cloning and Characterization of the Rotten Ear (*Rte*)/Truncated Inflorescence Development1 (*Tid1*) Gene in Maize

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Although the metalloid boron (B) has a relatively low natural abundance in comparison to other major elements, its role as an essential plant micronutrient should not be undervalued. In major crops such as maize, proper boron nutrition is critical for obtaining not only high yields but high produce quality as well. We identified a novel recessive mutant, called *rotten ear* (*rte*) /*truncated inflorescence development* (*tid1*) impaired in the development of both maize inflorescences, the tassel and the ear. *rte/tid1* mutants are completely sterile and fail to develop fully fertile inflorescences. Mutant *rte/tid1* ears appear to arrest during development and show a brown or "rotten" appearance. We mapped the *rte/tid1* mutant on chromosome 1.05 , and successfully isolated the *Rte/Tid1* gene by a map based cloning approach. Sequence and phylogenetic analyses suggest that *Rte/Tid1* encodes a putative boron transporter similar to the Arabidopsis thaliana *BOR1* gene, responsible for efflux transportation of boron. Although a notable phenotype was only detected in inflorescences, the *Rte/Tid1* gene is expressed in both vegetative and reproductive tissue. *in situ* hybridizations of *Rte/Tid1* in developing ears show localized expression in vascular tissue. Since the symptoms of boron deficiency are very diverse, with most of which accredited to secondary effect, it has been a challenge to determine the specific duties of boron in inflorescence and floral development. Because of this, we are interested in investigating the role of *Rte/Tid1* and boron during the development of maize inflorescences.

Funding acknowledgement: National Science Foundation (NSF)

P130

The *Sorghum bicolor ramosa1* locus impacts inflorescence architechture in transgenic maize and *Setaria*

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Expression of the maize *ramosa1 (ra1)* gene, which encodes a C2H2 zinc-finger transcription factor, imposes spikelet pair meristem (SPM) determinacy. In both the tassel and the ear of *ra1* mutants, long branches (LB) develop in place of spikelet pairs (SPs). Inflorescences of *ra1* mutants resemble wild type architectures of related grasses. We are interested in understanding functional relationships of *ra1* in plants with diverse inflorescence architectures. *ra1* is unique among members of the *ramosa* pathway: *ra1* orthologs have been identified only from Panicoid grasses, where SPs predominate as a morphological character. This group includes sorghum in addition to maize, and *Setaria* where spikelets are not paired. The maize *ra1* locus is comprised of a single gene copy; in transgenic maize plants containing 5.8kb of this locus, it complemented the tassel and ear phenotype of strong *ra1-R* mutants. The sorghum *ra1* locus contains a tandem duplication of *ra1*. However, a frameshift mutation disrupts the first copy; the second copy encodes a complete protein that is 69% identical to maize RA1. As a test of functionality, constructs containing either the entire sorghum locus, or only the functional downstream reading frame and flanking regions were generated and introduced into maize using *Agrobacterium*. The plants were examined for any impact on normal and *ra1-R* mutant inflorescence architecture. Analysis of inflorescences from T1 and T2 plants suggests that sorghum *ra1* confers SPM determinacy in maize. However, LB architecture in transgenic maize expressing sorghum *ra1* differs slightly from that of normal maize suggesting additional *cis* functions impart some sorghum-like characters to the inflorescence architecture. These data support the hypothesis that the sorghum *ra1* gene can provide function in maize. As a broader survey of evolutionary functionality, preliminary data of transgenic expression of maize and sorghum *ra1* genes in *Setaria* will also be presented.

Funding acknowledgement: National Science Foundation (NSF)

P131

The FT-like ZCN8 gene functions as a floral activator and is involved in photoperiod sensitivity in maize

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The mobile floral-promoting signal, florigen, is thought to consist of, in part, the FT protein named after the Arabidopsis thaliana gene Flowering locus T. FT is transcribed in leaves and its protein moves via the phloem to the shoot apical meristem where it promotes the transition from vegetative to reproductive development. In our search for a maize FT-like floral activator(s), seven ZCNs (Zea mays CENTRORADIALIS) genes encoding FT homologous proteins were studied. The ZCN8 gene has the requisite characteristics for having florigenic activity in maize. In photoperiod sensitive tropical lines, ZCN8 transcript was strongly up-regulated in a diurnal manner under floral-inductive short days. In day-neutral temperate lines, ZCN8 mRNA level was independent of day length and displayed only a weak cycling pattern. ZCN8 is expressed in leaf phloem cells and ectopic expression of ZCN8 in vegetative stage shoot apices induced early flowering in transgenic plants. Expression of the six other ZCN genes neither consistently correlated with the floral transition nor changed flowering time in transgenic plants. ZCN8 was placed downstream of indeterminate 1 (id1) and upstream of delayed flowering 1 (dlf1), two other floral activator genes. We propose a flowering model linking photoperiod sensitivity of tropical maize to diurnal regulation of ZCN8.

P132

The GRAS transkription factor *Brachytic crinkled leaf1* controls plant height, leaf angle and leaf morphology in maize

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Leaf angle, plant height and leaf morphology are key traits which influence yield in maize, irrespectively if yield is measured as harvest index or biomass production. In order to identify and study key regulators that control these traits we conducted forward genetic screens on *Mutator* transposon and EMS mutagenized maize populations. A monogenic, recessive mutant isolated from these screens was designated *brachytic crinkled leaf1* (*brc1*). *brc1* mutants reached about two thirds of wild-type height and produced leaves both shorter and wider than those of wild-type siblings. In addition, *brc1* plants showed an altered leaf angle resulting in upright leaves. To clone *Brc1* we used a combination of bulk segregant analysis (BSA) and AFLP analysis. We isolated a candidate gene for *Brc1* which shows homology to GRAS-like transcription factors. Using synteny mapping we identified the rice ortholog of *Bcr1*, whose mutant shares striking similarities to the *bcr1* phenotype.

P133

The PIN-FORMED family of auxin efflux carriers mediates auxin transport and accumulation shaping maize development

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Auxin regulates many aspects of plant development, such as embryogenesis, leaf, flower and lateral root initiation, vascular tissues differentiation and tropisms. In this context, PIN auxin efflux carriers play a key role in building up the spatial auxin maxima and minima (also called auxin gradients) that provide positional and directional information, required for the coordination of plant development. A better understanding of PIN role in mediating polar auxin transport (PAT) in maize is of outstanding importance for basic plant biology research and for crop improvement as well: shoot and root architectures, two of the main issues in determining crop yield, are indeed strictly regulated by PAT.

In the last years we identified three *PIN1* orthologs in maize, analyzed their expression patterns and protein localization during pre- and post-embryonic development, confirming the fundamental role of PIN1-driven auxin accumulation for proper monocots development.
Recently nine new *ZmPIN* genes were identified in our lab: an additional *PIN1* gene (*ZmPIN1d*), a *PIN2* gene, three putative orthologs of *AtPIN5* (*ZmPIN5a*, *ZmPIN5b* and *ZmPIN5c*) and the ortholog of *AtPIN8* (*ZmPIN8*). As previously reported in rice and sorghum, also in maize we identified three monocot-specific proteins (ZmPIN9, ZmPIN10a and ZmPIN10b). The study of *PINs* expression patterns at cell and tissue levels, focusing the attention on root system and kernel development, will be presented. In these tissues, IAA immunolocalization results reveal that auxin accumulation is strictly correlated with *PIN* expression. Furthermore, applications of the auxin efflux inhibitor N-1-naphthylphthalamic acid (NPA) seriously affect *PIN* expression, auxin accumulation and organ and tissues differentiation.

Taken together our results indicate that the more elaborated pattern of maize development compared to Arabidopsis is accompanied by a more complex PIN family organization with a highly regulated expression of its members.

P134

The developmental dynamics of the maize leaf transcriptome as revealed through ultra high throughput sequencing

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The maize leaf is an excellent model system to study the dynamic transition of a non-photosynthetic sink to a sugar-exporting source tissue. In maize, a gradient is established from leaf base (youngest) to leaf tip (oldest) enabling the capture of several developmental stages at a single time point. Like many of the world's most productive food and bioenergy grasses, maize utilizes C4 photosynthesis to harvest light energy and convert it into simple sugars. This specialized form of photosynthesis is achieved through a partitioning of photosynthetic activities between two cell types in the leaf. To develop a comprehensive framework for understanding C4 development in maize, we utilized Illumina deep sequencing to examine photosynthetic differentiation along a developing maize leaf and laser-capture micodissection to profile bundle sheath and mesophyll cells.

We observe that 64% and 21% of genes are differentially expressed along the developmental gradient and between mature mesophyll and bundle sheath cells, respectively. Cluster analysis of the data reveals an extremely dynamic transcriptome, with transcripts for cell cycle, primary wall synthesis and basic cellular metabolism at the leaf base transitioning to secondary cell wall biosynthesis and C4 photosynthetic development towards the tip. We implemented a web-based genome browser, an electronic fluorescent pictograph browser, and a two-cell biochemical pathway viewer to visualize the datasets. We demonstrate the value of this dataset for a systems biology approach to dissecting photosynthetic development.

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P135

The phytochrome interacting bHLH proteins affect photomorphogenic development in Maize

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The bHLH (basic helix-loop-helix) class of transcription factors has been shown to play key roles in the phytochrome signal transduction in Arabidopsis. Many of these transcription factors (e.g. PIF1, PIF3, PIF6 etc.) have been shown to possess an APB (active phytochrome binding) motif necessary for the interaction with phyB. We analyzed the latest maize genome sequence and annotation (5b.60) released by the Maize Genome Sequencing Project. We were able to identify at least 100 genes with the conserved bHLH domain. A small subset of these bHLH factors also contains the putative APB motif. Similar to Arabidopsis, we found that at least one of the bHLHs (ZmPIF3 Like) interacts with ZmphyB1 as shown by the yeast two hybrid and co-immunoprecipitation assay. Previously, we characterized the expression profile of multiple members of the maize bHLH gene family that are orthologous to the Arabidopsis PIFs, HFR1 and PIL1-like shown to be involved in light signaling. Several genes were found to be responding to red/far-red light in etiolated seedlings and to simulated canopy shade in de-etiolated plants. These results indicate that the protein-protein interactions of the phytochrome signaling mechanism are likely conserved between Arabidopsis and maize.

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P136

The quest for apomixis in Zea mays. Episode I: Raiders of the lost meiosis

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Apomixis is defined as asexual reproduction through seed (Nogler 1984). This phenomenon was reported for some species but is absent in crops. The production of seeds that are 100% identical to their mother is of tremendous agricultural potential to maintain desired genetic traits indefinitely. The main application of apomixis would be the maintenance of the heterozygous genetic state of hybrids, which would allow the usage of harvested kernels as seeds for the next season instead of relying on hybrid seed supply.
Our approach is to search for mutants displaying the main elements of apomixis: apomeiosis (the formation of egg cells with identical genetic constitution as their mother) and parthenogenesis (the development of an embryo without paternal genetic contribution). The combination of two mutants of these classes can result in clonal offspring (Koltunow 1993).
The work presented here is about the search and characterization of mutants displaying the first element of apomixis, the formation of egg cells with the identical genetic constitution as their mother (apomeiosis). The mutants were found in a screen based on the ploidy barrier in the maize endosperm (Lin 1984), by pollinating segregating *Mu* families by 4n^*R-nj* and screening for plump kernels displaying the *R-nj* pigmentation. The cross of *nrm4* and *nrm1/as1* with 4n^*R-nj* displayed the formation of viable diploid egg cells that maintained maternal heterozygosity, shown by ploidy and SSR analysis of the offspring. The confocal microscopic investigation confirmed the absence of normal meiotic divisions in these mutants.

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P137

Transcription profiles and associated histone modifications of genes encoding defensin-related proteins in the maize endosperm transfer cell

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Three principal cell types constituting the maize endosperm are basal endosperm transfer cells (BETCs), aleurone cells (ACs), and starchy endosperm cells (SECs). These cell types are morphologically distinct, spatially segregated, and play different roles during seed development. We have identified genes that are highly expressed in the BETCs, ACs, and SECs at 8 days after pollination (DAP), 12 DAP, and 16 DAP by means of micro-dissection and 454 pyrosequencing. As expected from their structural and functional dissimilarity, many genes were determined to be transcribed preferentially in one of the three endosperm cell types. A group of defensin-related protein (DEFL) genes are transcribed in massive amounts in the BETC at 8 DAP, accounting for ~40% of the total transcripts when estimated from their read numbers. Their transcription was exclusively confined to the BETC and rapidly diminished after 8 DAP. Spatial and temporal expression patterns of the DEFL genes were confirmed using real-time quantitative PCR and in situ hybridization. We hypothesized that regulation of the DEFL genes in the BETC involves modifications in the chromatin structure because promoter analysis of the DEFL genes indicated that they do not share common cis-elements and some of the BETC DEFL genes are clustered in the genome. We have detected histone modifications in the 5' regions of the BETC DEFL genes as well as known BETC-specific genes that are consistent with their transcriptional activities.

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P138

Using Natural Variation to Identify Gene Modifiers of Three Developmental Mutations in Maize

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Mutant phenotypes can vary when combined with different genetic backgrounds, mainly because of the presence or absence of gene modifiers. We are using the Nested Association Mapping (NAM) population, which are pre-genotyped recombinant inbred lines (RILs) derived from 27 diverse parents, to identify gene modifiers of three dominant developmental mutations, *Liguless3 (Lg3-O), Few branched1 (Fbr1)* and *Clumped tassel1 (Clt1). Lg3-O* causes small leaf angles (upright leaves) and understanding this genetic network can allow greater planting density. *Fbr1* alters lateral structure formation in tassels as well as structure identity, and *Clt1* leads to shortened and thickened tassels, but also shortened plant and leaf morphology. We have crossed these three mutations with a subset of the 27 diverse NAM parents to identify suppressors and enhancers of each. We present the variation observed in the F1 progeny of these crosses confirming that there are at least some dominant modifiers, and we will identify and map these using the F2 populations as well as crosses to the NAM RILs.

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P139

Using the *discordia* mutants to identify proteins needed for division plane orientation in plant cells

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In plants, cell wall placement at cytokinesis is determined by the position of the preprophase band (PPB) and the subsequent expansion of the phragmoplast, which deposits the new cell wall, to the cortical division site delineated by the PPB. New cell walls are often incorrectly orientated during asymmetric cell divisions in the leaf epidermis of the *discordia* (*dcd1*, *dcd2*, and *dcd3*) maize mutants. Cloning *dcd1* showed that it encodes an orthologue of the *Arabidopsis fass/ton2* gene, a putative B'' regulatory subunit that targets the serine/threonine phosphatase PP2A to appropriate substrates. An antibody that recognizes DCD1 and a closely related protein, ADD1, localizes these proteins to PPBs and, more surprisingly, the cortical division site that remains after PPB breakdown. Considered all together, these experiments suggest that phosphatase activity regulated by DCD1/ADD1 is needed for PPB formation and cortical division site establishment. To identify additional proteins needed for division plane orientation in plants and to expand our knowledge of DCD1 function, we are performing a yeast two-hybrid screen to find proteins that interact with DCD1. We are also using a map-based cloning approach to determine the molecular identities of the genes mutated in the *dcd2* and *dcd3* mutants. Progress on each of these projects will be reported.

Funding acknowledgement: University of North Texas

P140

Wart-like mutants that disrupt cell expansion during leaf development: Map-Based Cloning and Characterization of *Warty2* (*Wty2*)

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The maize leaf epidermis is useful for studying mechanisms of cell division and expansion because the cells are ordered and developmentally distinct. Altered cell expansion can be readily detected by screening leaves visually for protruding misshapen cells in the blade epidermis. We obtained a new recessive EMS mutant (courtesy of Tom Brutnell) that shows severe over-expansion of epidermal cells, causing the leaf to appear highly corrugated and wart-like, and is not allelic to the previously described mutant, *warty1* . Named *Warty2* (*Wty2*), the gene was mapped to chromosome 3L based on segregation of SNPs in a Mo17/B73 segregating population. Subsequently, the gene was primarily mapped within 2Mb between marker mCSU351 and UMC2581 in chromosome bin 3.06 by analyzing 348 F2 *Wty2* mutant segregants. To fine-map *Wty2* additional PCR-based makers were developed and 2262 F2 *Wty2* mutant segregants were analyzed. The gene was finally mapped between marker WT2M43 and WT2M14, in an interval of about 32 kb, which harbors two ORFs. DNA sequence analysis of the two ORFs in *Wty2* mutants uncovered a nucleotide transition mutation that resulted in a Gly to Glu substitution in the ATP binding domain of a putative Tyr kinase. To further confirm the gene identity, additional alleles are being generated using EMS mutagenesis, Ac/Ds mutagenesis lines and the UniformMu population. In addition, several transgenic lines are under investigation, including a complementation construct, over-expression and RNAi constructs, a *Wty2* promoter-GUS and a WTY2-YFP reporter line. The Plant Transformation Facility at ISU has transformed these constructs into maize, and analysis of the T0 plants is currently underway. Our initial observations indicate that YFP tagged WTY2 localizes to the plasma membrane in young dividing and expanding leaf tissue, as predicted. Final confirmation of protein localization, functional study and identification of new alleles is ongoing.

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P141

Widespread fasciation in the maize landrace Veracruz 85

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Meristems are responsible for organogenesis and for maintaining a pool of undifferentiated cells, which allows the meristem to maintain itself. Organ initiation fails to keep pace with meristem proliferation in the fasciated maize mutants *thick tassel dwarf1 (td1)* and *fasciated ear2 (fea2)*, which results in overproliferation of certain meristem types, most notably those involved in inflorescence development. The maize landrace Veracruz 85 displayed a variety of dramatic defects in both vegetative and inflorescence development including increased organ production and fasciation. Phenotypic characterization and developmental analysis of these plants suggest the most likely explanation for the observed phenotypes is an enlargement of all meristem types, which impacts all stages of maize development. Initial genetic mapping results suggest at least one candidate locus for the effects in bin 5.05 on chromosome five. No previously described mutant involved in meristem regulation maps to this region, but we found this chromosomal segment to be homeologous to a segment containing *fea2* on chromosome four. Using the collinearity between these segments and the orthologous regions in sorghum and rice, no obvious homeolog of *fea2* was found on maize chromosome five. However given the mapping results and synteny with the chromosomal segment containing *fea2*, gene(s) that contribute to these fasciated phenotypes in Veracruz 85 are likely located on chromosome five. Determining the genetic basis of the widespread fasciation in this accession could provide further insight into the regulation of meristem size in maize.

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P142

ZmAGO10 associated small RNAs and leaf polarity

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Members of the ARGONAUTE (AGO) family are key components of effector complexes involved in gene silencing pathways guided by small RNAs. Most plants encode multiple AGO proteins that form distinct complexes containing characteristic subsets of small RNAs, selected on the basis of their size and 5' nucleotide, and that thus possess unique biological activities. The *Arabidopsis* AGO family includes ten members. AtAGO10 has partially overlapping functions with AtAGO1, which mediates the effects of miRNAs on target gene expression. AtAGO10 is required for shoot apical meristem maintenance and functions in the establishment of leaf polarity. However, little is known about the pathways via which AGO10 regulates these processes.

A phylogenetic analysis showed that the maize genome contains twenty *AGO* family members, including two close homologs of AtAGO10. Transposon insertion alleles for both genes have been identified, and we are currently assessing possible contributions of the ZmAGO10 homologs to meristem function and leaf development. We also analyzed the small RNAs associated with ZmAGO10 by deep sequencing the small RNAs present in immunoprecipitates with a ZmAGO10 specific peptide antibody. We found that ZmAGO10 proteins preferentially bind 21-nt small RNAs containing a 5'-U, which are features common to nearly all plant miRNAs. However, the IP fraction was enriched for just a subset of known miRNAs and ta-siRNAs, and these include miR166 and tasiR-ARF, which we had previously shown to function in the specification of maize leaf polarity. A previously uncharacterized miRNA was most enriched in the IP fraction. *In situ* hybridization showed that this miRNA also accumulates in a polar pattern, establishing a gradient on the adaxial side of developing leaves. Screens for mutations affecting this miRNA and its targets are ongoing.

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P143

ZmEAL1 – a small protein secreted from the transition stage embryo

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ZmEAL1 (*EA1-like*) encodes a small protein precursor of 74 amino acids predicted to generate a mature secreted protein of 49 amino acids. Expression of *ZmEAL1* is restricted from the developing female gametophyte (FG) after cellularisation until the transition stage embryo. The finding that activity of the *ZmEAL1* promoter but no ZmEAL1-GFP-fusion protein is found in the suspensor of the developing embryo suggests that ZmEAL1 activity is strongly regulated at the post-transcriptional level. Secretion of ZmEAL1 seems to be regulated as well indicated by the presence of the protein in the endoplasmic reticulum, but not in the cell walls of the apical embryo region until the transition stage. Although its biological function is not fully understood, first knock-down phenotypes in the mature FG and during embryo development indicate a possible morphogen-like role of ZmEAL1 for cell fate determination and embryo patterning. Our search for interaction partners of ZmEAL1 in the developing maize embryo identified a number of AAA ATPases of unknown function(s). We are currently crossing various maker lines with ZmEAL1 expressing and mutant lines to study its role more precisely and to gain new insights into the fundamental processes of pattern formation in early embryo and FG development. These investigations are accompanied by gene expression analysis of endogenous *ZmEAL1* in BMS-suspension cells to study its regulation by the application of various phytohormons.

P144

Bulk Segregant map positions of UniformMu seed mutants can identify co-segregating transposon insertions

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Forward genetic analysis of seed mutants is limited by multiple factors. There are a large number of seed mutant loci with similar mature kernel phenotypes. The lethality of most seed mutants further impedes genetic analysis. Non-directed transposon-tagging populations can be used to identify co-segregating transposon insertions. However, co-segregation with dominant transposon-tags requires extensive population development prior to molecular analysis. To surmount these genetic challenges, we are mapping the *rough endosperm* (*rgh*) mutations identified from the UniformMu transposon-tagging population. The *rgh* mutant class displays an etched or pitted endosperm surface and four UniformMu *rgh* mutants are known to result from mutations affecting central metabolism enzymes or gene expression regulators. We crossed 144 rgh mutants to the B73 and Mo17 inbreds and are generating F2 mapping populations. Bulk Segregant Analysis (BSA) of nine rgh isolates identified seven map locations within 20 cM intervals. We co-localized these map positions with a collection of 5,000 non-redundant transposon flanking sequence tags (FSTs) from the same 144 mutants sequenced in a 2 dimensional grid. Four of the mapped mutants had candidate insertions that localized to the BSA map position. Preliminary segregation analysis of two insertions confirmed one co-segregates with the seed mutant phenotype.

P145

Molecular mapping of a novel *rough endosperm* mutant locus to the short arm of chromosome 5

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The primary food product of corn is the seed, and understanding the molecular genetics of seed development is of paramount importance for future crop improvement. We are focusing on *rough endosperm (rgh)* mutations identified from the UniformMu transposon-tagging population. The *rgh* mutant class displays an etched or pitted endosperm surface. So far, *rgh* phenotypes have been shown to result from mutations in genes affecting central metabolism and gene expression regulators. We are co-localizing map positions and transposon flanking sequence tags (FSTs) to clone additional *rgh* loci. Here we report the map position of rgh*-00F-064-09. This isolate was crossed to B73 to generate an F2 mapping population. Bulk Segregant Analysis of a pool of 24 rgh mutant individuals identified a segregation distortion peak on the short arm of chromosome 5. Individual recombinants were scored using public and custom simple sequence repeat (SSR) markers to map the locus to a 7.9 cM interval corresponding to 27 Mbp in the B73 reference genome. No other seed mutants have been mapped to the rgh*-00F-064-09 locus indicating this is a novel seed mutant. This map position was then compared to transposon FSTs for 144 rgh mutants sequenced in a 2 dimensional grid. Three candidate insertions were identified in the FSTs that corresponded to the row or column pools for rgh*-00F-064-09. None of these insertions corresponded to the rgh locus, and further mapping will be completed to more finely define the locus.

P146

Pericarp proteome reveals that *Unstable factor for orange1* plants exhibit altered expression of stress-associated genes

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In maize, the phlobaphene pigmentation in pericarp, cob glume, and tassel glume is controlled by maize pericarp color 1 (*p1*), a gene encoding an R2R3 MYB transcription factor (TF). *p1* induces accumulation of phlobaphene leading to tissue-specific expression. For example, *P1-wr* plants show white pericarps and red cob glumes differential expression patterns were attributed to both copy number and epigenetic modifications. Additionally, it has been shown that, in the presence of *Ufo1* (*Unstable factor for orange1*), *P1-wr* exhibit reduced DNA methylation. *P1-wr;Ufo1-1* ears show red pigmentation in both pericarps and cob glumes. In order to identify differentially expressed proteins in the pericarp tissue of *P1-wr;Ufo1-1* plants, isobaric tag for relative and absolute quantification (iTRAQ) was performed. As expected, many of the differentially expressed proteins identified through protein profiling are enzymes involved in the accumulation of flavonoids. Flavonoids are also well-known antioxidants induced to combat reactive oxygen species (ROS) during abiotic stress. Further, qPCR was used to validate the iTRAQ result and it showed that *MDAR* (monodehydroascorbate reductase), a gene involved in the recycling of ascorbate against ROS, was up-regulated by 3-fold in *Ufo1-1* pericarps. Additionally, primary metabolism such as glycolysis was also shown to be up-regulated, which provides substrates required for flanonoid production. Differential expression of genes correlates with stressed *Ufo1-1* plants and may explain linked phenotypes such as stunted growth and bent stem. Presumably, altered expression of these genes might be a consequence of over expression of P1. The fact that many of the differentially expressed genes are stress-inducible and have stress-responsive regulatory sequences suggests that *Ufo1* plants preserved stress memory despite the absence of stress. We will further confirm our iTRAQ results which show that several histone related proteins are affected in *P1-wr;Ufo1-1* plants. We are now interested in asking the question if epigenetic regulation is the upstream switch that is responsible for regulation of expression of stress associated genes in *P1-wr;Ufo1* plants.

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P147

A combination of RNA-Seq and ChIP-Seq reveals multiple roles of *P1* in pericarp development and metabolic pathways

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The maize pericarp is the outside layer of the seed coat, and it acts as the first barrier to insects and pathogens. It is also important for the quality of canned corn and popcorn as a moisture barrier. Depending on the genetic background,the pericarp can accumulate the phlobaphene pigments, derived from the polymerization of the 3-deoxyflavonoids and controlled by P1 (Pericarp Color1), corresponding to R2R3-MYB transcription factor. P1 also controls the accumulation of insecticidal C-glycosyl flavones in silks. While the metabolic steps resulting in the formation of a common precursor for the formation of the phlobaphenes and C-glycosyl flavones are known, most of the later steps in the respective pathways remain unidentified. To date, only the A1 gene, encoding dihydroflavonol reductase (DFR), has been confirmed as an immediate direct target of P1. In order to establish the overall regulatory function of the P1 gene, we performed a genome-wide analysis by a combination of RNA-Seq and ChIP-Seq in P1-rr, specifying red pericarp and red cob glume color and P1-ww, which harbors a null P1 allele lacking phlobaphene pigments. We successfully obtained short read sequence tags from mRNA and ChIP DNA extracted from pericarp cells of P1-rr and P1-ww at two different developmental stages, 15 and 25 DAP. The computational analysis of RNA-Seq and ChIP-Seq data revealed that P1 particularly plays a role in the flavonoid pathway, and to a lesser extent in the phenylpropanoid pathway. It also suggested that P1 could be part of other biological processes. This is the first report of the comprehensive analysis that integrated the information of gene expression changes and binding locations of a tissue specific transcription factor in maize. This project was funded by grant NSF DBI-0701405.

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P148

A novel strategy for mapping opaque2 modifier genes with γ-irradiation mutagenesis

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Quality Protein Maize (QPM) was developed by breeding hard kernel, high-lysine variants of the opaque2 mutant. Various mapping experiments have identified major QTLs for modification of the soft kernel endosperm phenotype to an agronomically useful vitreous kernel form. Furthermore, transcriptional profiling experiments have identified a number of candidate genes that could account for or contribute to these QTLs. In order to fine map the QTLs and potentially identify other regions containing o2 modifier genes, we are using a γ-irradiation mutagenesis approach. 2000 kernels of a QPM line developed in South Africa, K0326Y, were mutagenized and propagated in the field. After loss of plants due to reduced germination and plant fitness resulting from the radiation treatment, approximately 300 variably filled M2 ears were recovered. Screens of well filled M2 ears have identified a number of opaque revertants and we will present preliminary phenotypic characterization. In addition to resumption of the opaque phenotype, several of these lines have low levels of γ-zein. γ-zein is an essential component of o2 modification and a candidate for one of the major QTLs. We are selecting markers to test for chromosome deletions both within the QTL coordinates and across the genome. We are propagating the population to the M3 and this generation will be further screened for kernel as well as whole plant phenotypes.

P149

Advancing complex phenotype analyses through machine vision and computation

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Phenotyping methods frequently limit functional genomic studies. Phenotypes are currently not studied with the same degree of sophistication or throughput as genomes or processes more proximate to the genome such as gene expression. Because phenotypes are such an important source of information about gene function, we are integrating multiple machine vision platforms to study seed and seedling phenotypes. Machine vision utilizes information contained in an image or other optoelectronic signal, such a reflectance spectroscopy, to collect quantitative measures of phenotypes. We are 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, kernel color and 3D shape, and dynamic seedling root growth. All of these phenotypes are collected for individual kernels providing greater statistical power to detect interrelationships that have a physiological basis. Computational workflows are being developed to automatically extract biologically relevant data from each phenotyping platform and to interrelate the machine collected data. We are using this pipeline to identify quantitative trait loci (QTL) underlying seed and seedling phenotypes within the maize Nested Association Mapping (NAM) population. Preliminary studies with the NAM parents suggest that seed composition traits are interrelated to seedling root gravitropism responses.

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P150

Analysis of Chromatin Accessibility in the Maize Interphase Nucleus

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Nuclease sensitivity has long been used to characterize the accessibility of chromatin at specific loci to soluble nuclear factors, and is associated with transcriptional activation. Performing nuclease sensitivity assays with microarray technology allows for a genome-wide analysis of chromatin structure in maize. To understand the functional significance of chromatin accessibility in the interphase nucleus, three-dimensional fluorescence microscopy will be employed to visualize the spatial organization of highly accessible DNA relative to highly inaccessible DNA, the nuclear envelope, and epigenetic features of active chromatin. Chromatin structure changes in response to development, environmental stimuli, and mutations in chromatin-associated genes. We are developing this technology in order to examine the relationship between changes in chromatin structure and changes in spatial organization. To explore changes of specific foci, cytogenetically mapped Sorghum BACs will be used as FISH probes. This work will establish a new platform for the analysis of how the maize genome regulates global and local chromatin structure under various conditions, while shedding light on the functional organization of the maize genome in the interphase nucleus.

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P151

Assay conversion efficiency of single nucleotide polymorphisms released by Beijing Genomics Institute

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Despite the availability of a large number of SNP candidate sequences in the public domain, it was previously shown that only a small portion of those polymorphisms could be converted into viable SNP assays and used in molecular genetics and breeding projects. In this study we tested the assay conversion efficiency of a subset of recently released SNP candidate sequences from Beijing Genomics Institute (BGI). In silico characterization of a small subset of BGI SNPs revealed that ~70% of the sequences represented repetitive and duplicated regions of maize genome, which do not meet the sequence requirement of modern genotyping chemistries such as GoldenGate, Infinium, Taqman and KASPar. Remaining single copy SNP candidate sequences were used to design KASPar assays with ~80% success rate. Minor allele frequencies of successfully validated SNPs were above 0.3 indicating the highly polymorphic nature of these SNPs. Developed SNP assays can be leveraged in the characterization of North American germplasm.

P152

Control of phenylpropanoid biosynthesis in maize

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Grasses, including maize, are a major source of agricultural biomass, offering significant opportunities for increasing renewable fuel production. The efficiency of biofuel production is influenced by lignin content which can significantly reduce the amount of extractable sugars. To explore the possibility of altering lignin content we are investigating regulators of the phenylpropanoid pathway. In maize, C1 and P1 are both members of the R2R3-MYB class and control different branches of the phenylpropanoid pathway. Based on the mechanisms by which P1 and C1 control gene expression, we hypothesized that closely related R2R3 MYB^{PtoA} members would control other branches of the phenylpropanoid biosynthetic pathway. *ZmMYB40* (aka *ZmMYB-IF35*) and *ZmMYB95* (aka *ZmMYB-IF25*) are two members of the R2R3-MYB^{PtoA} group and they express in most plant tissues, and *in situ* mRNA hybridization showed their expression strongly associated with vascular bundles. Both of these MYBs have been identified as positive regulators and ZmMYB40 binds the high-affinity P1-binding site in the A1 promoter. Distinct from P1, however, *ZmMYB40* overexpression in maize cells induces the accumulation of phenylpropanoids (ferulic acid, chlorogenic acid, and caffeic acid), but not flavonoids, suggesting it regulates a different branch of the phenylpropanoid pathway. In order to identify direct targets for ZmMYB40 and ZmMYB95, maize transgenic lines harboring RNAi constructs to silence the respective genes have been generated. We have identified lines showing decreased ZmMYB40 or ZmMYB95 expression that will be used for cell wall composition analyses in addition to target gene expression analysis. Characterization of these MYBs in combination with investigation of negative regulators of lignin biosynthesis (ZmMYB31 and ZmMYB42) will help explain the intricate regulation of lignin and lignin precursor production in maize cells. These studies will also help identify approaches for genetic improvement of maize and other important grass species as feedstocks for biofuel production.

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P153

Discovering the Roles of miRNAs Rapidly Responding to Short-term Submergence in Maize Root Cell

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Waterlogging leads to low oxygen levels (hypoxia) in the plant root zone causes a metabolic switch from aerobic respiration to anaerobic fermentation that results in rapid changes in gene transcription and protein synthesis. Our work seeks to characterize the post-transcriptional gene regulatory networks associated with the waterlogging. MicroRNAs (miRNAs) are small non-coding RNAs that regulate many genes involved in growth, development and various biotic and abiotic stress responses. To characterize the involvement of miRNAs in response to hypoxia conditions, we did small RNA profiling experiments. High-throughput sequencing of small RNA libraries obtained from 4h waterlogging-treated and control root tissue identified a total of 72 unique miRNA sequences from 28 miRNA families in Hz32, which is the most tolerant inbred line to waterlogging based on our pervious study. Hypoxia resulted in changes to the abundance of 22 miRNAs from 18 miRNA families. We computationally predicted the targets and the stress related cis-regulatory elements on the promoters of these miRNA families. We used Q-PCR to confirm the expression changes of these miRNAs and their predicted targets in 3 inbred lines that have different sensitivity to waterlogging. This work suggests that miRNAs play important role in early responses to hypoxia conditions and gene regulation.

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P154

Disease-resistant Mechanism to Head smut of maize Based on DGE and Microarray

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The maize head smut is caused by the Sporisorium reilianum f. sp. Zeae, which infect the maize by forming the dicaryotic hyphae to penetrate the root at the early seedling period. In China, particularly in the maize producing areas, the yield loss is up to 300,000 tons due to this disease each year.
The resistance to head smut disease for maize mainly depend on the resistibility to hyphae at two growth stages of this pathogen: invasion stage and proliferation stage. In our studies, Digital Gene Expression (DGE) and Maize 70-mer Oligo nucleotide arrays (Microarray) were respectively used to assay responsive genes to infection and proliferation in two distinct resistance lines (Huangzao4 and Mo17).
When hyphae begun to infect on the epidermis of roots, there were 256 significantly different expressed genes (110 up, 146 down) in Mo17 and 156 significantly different expressed genes (91 up, 65 down) in Huangzao4. The Mo17 showed the more defensive response and less nitrogen utilization activity than Huangzao4. When the hyphae invaded into the roots, there were 127 significantly different expressed genes (122 up, 5 down) in Mo17 and 410 significantly different expressed genes (78 up, 332 down) in Huangzao4. The genes with O-methyltransferase activity, which is the key enzyme in the pathway of Lignin synthesis, were significantly down-regulated in Huangzao4, but up-regulated in Mo17. It was proved that the lignin deposition accumulated in roots of Mo17 but decreased in roots of Huangzao4.
When hypae begun to grow in the body of host, they grew by way of intercalary hyphal extension so as to keep harmonious relationship with the host, then proliferated intensively and broke the cells when the growth of host begun transform from vegetative stage to reproductive stage. There were less different expressed genes in Mo17 at the v2, v3 and v6 stage. However, 235 significantly different expressed genes (174 up, 51 down) were found at the early v9 stage. Our data indicated that the early v9 stage played an important role in the resistance to proliferation of hyphae by regulating the crosstalk among auxin, flavonoid and ABA.

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P155

Distribution of Recombination Frequencies across Maize Chromosomes

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We leveraged a high-density maize single nucleotide polymorphism (SNP) map and the physical map of B73 reference genome to identify the frequency of recombination events across maize genome. Understanding the distribution of recombination frequencies in maize genome is valuable for positional cloning of gene/QTL and identification of optimal number of SNPs necessary for the genetic analysis of a particular region of maize genome. In this study we identified the areas with low (≥ 6 Mb/cM), medium (between (1 Mb/cM to 6.0 Mb/cM) and high recombination frequencies (≤ 1 Mb/cM). We were able to delimit the borders of centromeres in all 10 chromosomes and in some chromosomes the putative positions of alternative centromeres were also discovered.

P156

Expression profiling and evolution of pathogenesis related genes in maize and teosinte in response to *Ustilago maydis*

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In nature there is a constant arms race between the attacking parasite and the defending host. The co-evolution of plants and their corresponding pathogens has substantially reduced plant disease control strategies. One of the most convenient, inexpensive and environmentally sound ways to control plant disease is to utilize disease resistant varieties. Maize *(Zea mays)*, a domesticated form of teosinte is a globally important agricultural crop and a classical system for studying genome structure, function and heritability. Molecular dating indicates that maize and teosinte diverged approximately 9000 years ago, a date that agrees well with archaeological evidence. Interestingly, both maize and teosinte are able to be infected by corn smut *(Ustilago maydis)* a fungus which turns infected grains into rapidly growing, often large and shapeless galls. We inoculated maize and teosinte with a strain of *U. maydis* and found that two teosinte lines *(Zea diploperennis* and *Zea luxurians)* exhibited a high level of resistance and were similar to maize in phenotypic response. These data and the morphological and genetic similarity between maize and teosinte indicate maize and teosinte may share key components in plant defenses and the activation of pathogenesis related (PR) genes. Therefore, we will identify and characterize PR genes that are differentially expressed in maize, teosinte and teosinte x maize introgression lines (NILs) in response to *U. maydis* infection. These studies will provide incite as to the expression pattern, genetic diversity and evolution of these types of genes in maize and its wild progenitor teosinte. This information will be used to potentially identify other sources of resistance to *U. maydis* and assist in the design of novel strategies to develop maize cultivars with high levels of resistance to U. maydis as there are no maize lines with strong resistance to this fungus.

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P157

Free amino acid profiles as a functional marker for studies on nitrogen metabolism in maize

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Nitrogen is one of the most important nutrients and is often the limiting factor for plant growth. For these reasons, farmers supplement far more nitrogen to their fields than any other nutrient. Maize is an excellent and proven model for the studies on nitrogen use efficiency (NUE). Improvement of maize NUE is one of the much sought after objectives as maize is the major food crop grown in the United States which requires optimal amount of nitrogen to maximize yield. Several agronomic and genetic studies led to the identification of various traits, QTL and genes associated with NUE. Free amino acids are the currency for plant N metabolism, and thus may be a potential metabolic marker correlated with maize N utilization. We have identified allelic variants in asparagine synthetase (AS) and asparaginase (ASNase) genes that may contribute to genetic variation for NUE. We tested the effects of these alleles on free amino acid accumulation in earshoots of diverse inbreds and hybrids grown under N-limiting or N-sufficient conditions. Developing maize ear shoot was used as the experimental system. Phenotypic traits such as grain yield, nitrogen content and stover biomass were measured and their correlations were observed against free amino acid profiles. Plants grown under sufficient nitrogen showed marginal increase in total free amino acids. Asparagine and Glutamine showed significant increase with the application of nitrogen. Allelic composition showed characteristic amino acid profiles especially the amino acids that participate in asparagine cycling pathway.

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P158

Future Developments for Non-Destructive 3D Plant and Root Imaging

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High-throughput screening and high-throughput phenotyping have become key technologies for research in and development of active ingredients for pharmacology, new plant protection compounds and breeding for new traits in agricultural products. These technologies are fundamentally important for many fields of applied and basic research, enabling the examination and understanding of different plant gene functions and the overall effects of chemicals on various organisms. Most of these screening methods are measuring visible parameters of the plants such as colour, shape, size, area, architecture, growth rate, performance or movement. Therefore digital imaging of plants has become a very important tool in plant research, since modern image processing software algorithms are much better and more reproducible in quantifying these visual parameters than the human eye. Moreover the spectrum of modern CCD cameras can be extended to lower or higher wavelengths far beyond the visual range of the human eye such as Near Infrared (NIR) for measuring the water distribution and dynamics in plants during drought stress experiments. However all this reflective measurements are just able to target the visible part of the plant, the shoot, while the root keeps to be hidden in the soil or substrate. The goal of this joint study is, to explore weather Nuclear Magnetic Resonance Imaging (NMRI) or (Sub) Terahertz Imaging (THz) might be used for obtaining non invasive and valuable information about plant roots in soil or substrate.

P159

Gene-dose dependent control of seed mass by endosperm-specific *Miniature1 (Mn1)*-encoded cell wall invertase (CWI), which also affects embryo mass and embryo sugar physiology

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The *Mn1* locus is a major determinant of sink strength of developing seeds through its control of both sink size, i.e., cell size and cell number, and sink activity via sucrose hydrolysis and the release of hexoses essential for energy and signaling functions. Hexoses are also essential for the development of basal endosperm transfer cells (BETCs), the sole gateway for sugars and other nutrients from the pedicel. Not surprisingly, loss-of-function mutations at the *Mn1* locus lead to the *mn1* seed phenotype that show ~ 70% reduction in seed mass at maturity and several pleiotropic changes, including the altered levels of auxin, IAA, and cytokinins in the developing endosperm. Previously we reported an EMS-induced semi-dominant allele, *mn1-89*, showing higher levels of the *Mn1* RNA but greatly reduced CWI activity (~6% of the WT); however, the seed phenotype is near-normal. We show here that seed mass in the *mn1-89*, its reciprocal hybrids with *mn1-1*, and the *mn1-1* (a null allele) was dependent on the number of *mn1-89* alleles in the endosperm. The *Mn1* RNA levels were also gene-does dependent by q-PCR analyses; a similar correlation with the CWI activity is shown previously (Plant Cell 8: 971). However, no such correlation was seen in the levels of IAA and a major cytokinin, zeatin, in 12 DAP endosperm

A comparative analysis of endosperm and embryo mass in the *Mn1* and *mn1-1* at 12, 20 and 28 DAPs showed significant reductions of both tissues in the *mn1-1* for all three stages. Clearly, embryo development was endosperm-dependent. To gain a mechanistic understanding of this interaction, sugar levels were measured in these samples. Levels of all three major sugars, glc, fru and suc, in the embryos reflected a pattern similar to its endosperm, indicating a metabolic-dependence or -cross-talk between endosperm and embryo despite the well documented evidence of their genetic autonomy in sugar / starch pathways.

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P160

Generating complete physical maps of maize centromeres

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The centromere-specific histone H3 variant CENH3 is the universal epigenetic mark of centromeric chromatin. The centromeric satellite repeat (CentC) and centromeric retrotransposons (CRM) are enriched at all ten maize centromeres yet their role in centromere function remains unknown. We previously characterized centromeres 2 and 5 of maize. Centromere 2 contains a single 1.8 Mb CENH3 binding domain that is enriched in CRM1 and CRM2, and contains a CentC cluster near the middle of the domain. Centromere 5, in contrast, has two CENH3 binding domains, one of which (3.2 Mb) is enriched for CRM2 and contains CentC at the periphery of the domain, and a second one (1.0 Mb) that lacks centromeric repeats. These domains are separated by a 2.8 Mb CRM1-rich interstitial region. To learn more about the arrangement of centromeric repeats on other centromeres, we have delineated the functional centromeric regions on RefGen_v2 (viewable in MaizeGDB) by mapping reads that had been immunoprecipitated using anti-CENH3 antibody. We have also sequenced several CentC-rich singleton BACs (i.e., BACs that were not placed into FPC contigs in the initial physical map) at 6X to 10X coverage and ~100 centromeric repeat containing singleton BACs at 1X coverage, and are in the process of placing them on the physical map. Using these and other resources we plan to close as many centromeres as possible. A comparison of centromere 10 with the previously finished centromeres 2 and 5 will be provided.

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P161

Genetic architecture of maize and teosinte

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Genetic architecture is the constellation of gene effects and interactions that underlie variation in a quantitative trait. Essentially, genetic architecture is the map between phenotype and genotype. Understanding variation in genetic architecture is key to understanding evolution, manipulating species for a sustainable agriculture, and preserving variation as species adapt. This NSF project (DBI 0820619) is improving our understanding of the genetic architecture of complex traits in maize and its wild relative, teosinte. Maize has a combination of life history, economic and societal value, and genetic tools that make it uniquely suited to studying genetic architecture. We are identifying genes that control domestication traits and three key agronomic traits: flowering time, plant height, and kernel quality. Genetic linkage, association, and fine mapping analyses are being performed on the largest and most diverse set of mapping families publicly available for any species. A large series of isogenic lines are being used to characterize allelic series and epistatic interactions. The genetic architecture of each of the four trait groups will be compared and contrasted, and the influence of recombination and past domestication bottlenecks on the genomic distribution of functional diversity will be examined. Finally, the ability of genetic architecture-based models to predict phenotype will be evaluated in a broad range of germplasm, including elite US hybrids. This project will take a step toward the ultimate goal of predicting phenotype from genotype.

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

P162

Genetic variation for regulation of gene expression associated with N remobilization in maize

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Maize (*Zea mays*) is a C4 plant with a high capacity to uptake, assimilate and remobilize inorganic nitrogen (N) which is often limiting for biomass accumulation and grain yield. Efficient N utilization depends upon the coordinated regulation of physiological processes that mediate the remobilization of stored N within leaves to developing seeds. A better understanding of gene expression programs that mediate N remobilization would provide tools for improving grain yield under low N input. Amino acids and carbohydrates represent major metabolites transported from source (leaves) to sink (developing leaves and earshoot) tissues. Compared to carbohydrates such as sucrose, the coordinated changes in N metabolism that occur during assimilate remobilization have been less studied. We have compared free amino acids profiles (an indicator of N remobilization), expression of key N and C related genes, and partitioning of N and biomass among genotypes that vary for N utilization. These genotypes were grown with different levels of N supply in the field. B73 and Illinois Low Protein1 (ILP1) show high N utilization, Mo17 intermediate, and Illinois High Protein1 (IHP1) low N utilization. Leaves and earshoots were sampled from each genotype and N treatment at twelve time points that span the period of active N remobilization. Our amino acid profiles revealed that amino acid biosynthesis and accumulation is developmentally regulated, with significant qualitative and quantitative differences observed among genotypes for both leaves and seeds. The developmental changes in amino acids were coordinated with interesting changes in the expression of many N and C metabolism related genes. The anaplerotic, asparagine recycling and sucrose biosynthetic pathways were among pathways that exhibited the strongest associations with N response and N remobilization differences among genotypes. The observed metabolic and transcriptional changes were correlated to N partitioning and biomass allocation among different plant organs. Our results demonstrate that genetic variation for N utilization is programmed by coordinated changes in the regulation of gene expression that shift leaves from the accumulation of N to its remobilization, with high grain yield being associated with maintaining N in metabolically active forms.

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P163

Genome Dosage Effects on Heterosis and Gene Expression in Triploid Hybrids of Maize

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Heterosis is the phenomenon that hybrid progenies perform better than both inbred parents for characters such as plant biomass, development rate and fertility. To test the genome dosage effects on heterosis and hybrid gene expression, triploid hybrids derived from B73 and Mo17 that differ in their genome dosages were analyzed. Heterosis was evaluated by measuring nine characters (4-week and 6-week height, adult height, leaf length and width, ear length, tassel branch number, days to anther and silk emergence) in reciprocal triploid and diploid hybrids, Mo17/B73 (MBB and MB) and B73/Mo17 (BMM and BM), as well as the corresponding inbreds, B73 (BBB and BB) and Mo17 (MMM and MM). High-parent heterosis was found in both the reciprocal triploid and diploid hybrids for most characters. Correlated with the fact that B73 is better than the Mo17 inbred for most of the measured traits, the MBB hybrid performed better than the BMM, while the two diploid hybrids lack such a relationship. Furthermore, the two reciprocal diploid hybrids did not differ significantly for eight of the measured characters, while significant differences were found between the two reciprocal triploid hybrids for six of the measured characters. These results suggest that genome dosage affects heterosis in triploid hybrids. Gene expression in triploid hybrids is also influenced by genome dosage. Microarray analysis of gene expression in adult leaf using the maize 70-mer oligo array revealed 4,284 and 6,480 non-additively expressed genes in the BMM and MBB hybrids, respectively; 2,200 genes exhibit non-additivity in both the triploid hybrids. Among 1,989 non-additive genes that can be clearly categorized as dominant or over-dominant, 48.77% are dominant; 34.84% are over-dominant in one hybrid; 16.39% are over-dominant in both hybrids. The degree of non-additivity for gene expression is correlated with the genome dosage in triploid hybrids but not the parent of origin.

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P164

Global gene expression profiling in normal and interploidy endosperms

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In maize, as in other angiosperms, double fertilization generates the zygote and the primary endosperm nucleus, which is triploid due to the fusion of the two polar nuclei with one haploid sperm. A normal endosperm has a 2:1 maternal to paternal genome ratio. However, this is altered in interploidy crosses (e.g., diploid x tetraploid or tetraploid x diploid) and because maize interploidy endosperms eventually abort, these seeds do not germinate. In order to study the underlying causes of this phenomenon, we utilized Illumina deep sequencing on RNA isolated from maize OH43 endosperms of diploid, tetraploid and interploidy (2n x 4n and 4n x 2n) crosses collected at 10, 12 and 14 days after pollination. Total RNA was isolated to generate mRNA libraries and Illumina single-end sequencing performed. This produced approximately 12 -15 million 42 base reads per sample. Using the B73 maize reference genome, short sequence alignment was performed and read abundance per transcript measured. The data were modeled using a Poisson distribution, and genome-wide transcript differential expression statistically tested within each genotype across days. From more than 100,000 transcripts that were statistically tested, 2900 transcripts were significantly differentially expressed. A majority of these transcripts were found to be highly expressed in 2n x 4n endosperms compared to other genotypes at the 14th day after pollination. These results are expected since the endosperm proliferation phase is extended in 2n x 4n endosperms. Our analysis also revealed that transcription of certain transposable elements is specifically silenced in 2n x 4n endosperms compared to normal endosperms.

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P165

Identification and Characterization of Maize MicroRNAs Involved in the Very Early Stage of Seed Germination

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MicroRNAs (miRNAs) are a new class of endogenous small RNAs that play essential regulatory roles in plant growth, development and stress response. Extensive studies of miRNAs have been performed in model plants such as rice, Arabidopsis thaliana and other plants. However, the number of miRNAs discovered in maize is relatively low and little is known about miRNAs involved in the very early stage during seed germination. In this study, a small RNA library from maize seed 24 hours after imbibition was sequenced by the Solexa technology. A total of 11,338,273 reads were obtained. 1,047,447 total reads representing 431 unique sRNAs matched to known maize miRNAs. Further analysis confirmed the authenticity of 115 known miRNAs belonging to 24 miRNA families and the discovery of 167 novel miRNAs in maize. Both the known and the novel miRNAs were confirmed by sequencing of a second small RNA library constructed the same way as the one used in the first sequencing. We also found 10 miRNAs that had not been reported in maize, but had been reported in other plant species. All novel sequences had not been earlier described in other plant species. In addition, seven miRNA* sequences were also obtained. Putative targets for 106 novel miRNAs were successfully predicted. Our results indicated that miRNA-mediated gene expression regulation is present in maize imbibed seed.

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P166

Identification and characterization of new brown midrib genes in maize

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Brown midrib mutants in maize are known to be associated with reduced lignin content and altered lignin composition, and increased cell wall digestibility, which leads to better forage quality and higher cellulosic conversion efficiency into ethanol. Four well known brown midrib mutants in maize, named bm1-4, have been identified several decades ago. Besides these 4 characterized genes, 13 additional spontaneous mutants named bm*A to bm*M show brown midrib appearance. Allelism tests revealed three new brown midrib loci, which were designated as bm5, bm6, and bm7. Among them, bm5 includes bm*E, bm*F, bm*G, and bm*H allelic mutations. Bm6 and bm7 were each only represented once in mutant stocks bm*J and bm*I, respectively. Mapping studies for all three genes are ongoing (bm5 in the lab of Dr. Patrick Schnable). We currently focus on genetic mapping of bm6, which has been located to an interval spanning about 180kb on Chromosome 2. Additionally, we are in the process of evaluating the effects of these new bm mutants on forage quality, lignocellulosic ethanol conservation, and biomass by use of isogenic lines. Our study will provide tightly linked markers for marker aided selection in relation to forage quality and lignocellulosic ethanol conversion of stover, and will greatly advance our knowledge of cell wall lignifications after isolation of the underlying genes of these novel bm genes.

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P167

Identification of the Gene Products Responsible for Maysin Biosynthesis in Maize

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One of the most damaging insect pests that affect maize production is corn earworm [*Helicoverpa zea* (Boddie)], responsible for annual harvest losses of 2.5% (http://ipm.illinois.edu). In order to decrease the devastating effects that this insect has in corn production, farmers may have to apply pesticides up to 30 times per season, increasing production costs and also the health risks associated to excessive application of pesticides. Compounds responsible for conferring natural resistance against this pest belong to the flavonoid family of specialized compounds, more specifically, the C-glycosyl flavones maysin, apymaysin and methoxymaysin, which accumulate in maize silks where they carry out their biocidal action when ingested by the worm (Byrne et al, 1996). The main focus of this research is on the biosynthesis of maysin, in which the R2R3-MYB transcription factor P1 (or its duplicate P2), along with the *salmon silk* loci *sm1*, *sm2* and *recessive enhancer of maysin, rem*, have been associated with the accumulation of this C-glycosyl flavone in maize silks (McMullen et al, 2004). The *sm* maize mutants display salmon silks and browning color after cutting, yet only in the presence of the transcription factor P1/P2, supporting the involvement of this transcription factor in the biosynthesis of these C-glycosyl flavones (Grotewold et al, 1998; Byrne et al, 1998). The identity of *sm1* and *sm2* is not known, but mutant analysis suggested that *sm2* may have rhamnosyl transferase activity, and *sm1* be involved in the last step of maysin biosynthesis.

We will present here our efforts searching for *sm1* and *sm2* candidate genes by contrasting the mRNAs (analyzed by RNA_Seq) represented in *P-rr* and *P-ww* pericarps. Our goal is to determine and characterize the products of the *sm1* and *sm2* loci using analytical chemistry, biochemistry and molecular biology techniques in order to increase our knowledge of the flavonoid biosynthetic pathway. This Project was funded by grant NSF DBI-0701405.

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P168

Identifying Candidates for the Restorer-of-Fertility Gene *Rf3* in Maize CMS-S

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Cytoplasmic male sterility (CMS) is a maternally inherited trait that prevents normal pollen development. The pollen grains develop until the point of maturity before undergoing rapid disintegration and collapsing at the starch-filling stage. The expression of chimeric mitochondrial transcript *orf355-orf77* has been associated with sterility in CMS-S (Zabala et al., 1997). Cleavage of *orf355-orf77* by the nuclear restorer gene *Rf3* results in reversing male sterility in CMS-S (Gallagher et al., 2002). Since CMS-S has gametophytic restoration, a dominant *Rf3* allele is needed for fertility. If a plant is heterozygous (*Rf3/rf3*) half of the pollen is viable while the other half of the pollen is not functional. *Rf3* has been localized 4.3 cM distal to *white pollen1* (*whp1*) and 4.6 cM proximal to *BNL17.14* on the long arm of chromosome 2 (Kamps and Chase, 1997). The goal of this project is to identify *Rf3* using a candidate gene approach. A variety of organelle targeting programs was automated to search for genes on chromosome 2L that could encode mitochondrial proteins. Additionally, searches for genes with pentatricopeptide repeats (PPR) in the same regions were conducted. These criteria for selecting candidate genes were chosen because all known CMS restorer genes, except for one, are mitochondrially targeted PPR proteins. Additionally, the candidate genes were subdivided into specific classes of PPR proteins since restorer genes are known to be P class PPR proteins. Six candidate genes were PCR-amplified and sequenced in several *Rf3*-containing restorer lines and non-restoring *rf3* lines. Further analysis is underway to determine if any of these candidates are likely to be *Rf3*.

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P169

Illuminating Maize Biology: Investigating functional genomics in maize using tagged-fluorescent protein lines

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With the release of the B73 genome sequence, maize has become a powerful model system for functional genomics in the grain crops. Nevertheless, new methods of investigating gene function within cells and tissues are still needed to understand protein function at the cellular/subcellular level. To meet this need, we are generating 100 stable, natively expressed, fluorescent protein (FP) fusion lines to provide a useful molecular resource for the research community. The lines we have developed have provided new views of maize cellular architecture and we can now study the localization and real-time dynamics for over 90-tagged proteins. These lines mark most major cellular compartments and can be used to study developmental and physiological processes, including hormone signaling and vesicle trafficking. Data on the characterization of these lines, including confocal micrographs and movies, are accessible on the website, http://maize.jcvi.org/cellgenomics/index.shtml. The website also includes a community submission form to request genes for tagging.

We are now developing tools to optimize the LhG4 2-component transactivation expression system for use in maize. Selected and requested maize promoters will be used to drive tissue-specific expression of the LhG4 transcription factor, which in turn will transactivate genes cloned downstream of pOp sequences. The project will make use of new maize optimized fluorophores to label subcellular compartments and will develop techniques for live cell imaging of growing maize shoot apices and leaf primordia. The project will deliver a permanent stock of stably transformed seeds for 50 promoter/driver lines, 20 new FP-tagged lines, fully characterized in vivo imaging methods, gene and reporter constructs, and a robust pipeline for handling large image datasets. Image and metadata will be processed using the bioimage database management system, Bisque. Images and metadata will be migrated to the community database MaizeGDB annually and seeds will be distributed to the Maize Stock Center.

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P170

Isolating Resistance Genes against Sugarcane Mosaic Virus in Maize

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Sugarcane mosaic virus (SCMV) is one of the most important virus diseases in maize worldwide. Susceptible cultivars infected by SCMV often develop symptoms like stunting, chlorosis, reduction in plant weight, and therefore, reduction in grain and forage yield. Previous studies revealed two major resistance loci conferring resistance to SCMV, one located on chromosome 3 (*Scmv2*) and one on chromosome 6 (*Scmv1*). Two isogenic mapping populations that segregating in one of the resistant loci, but not in the other, were developed respectively to minimize genetic variation potentially affecting expression of SCMV resistance. Based on publicly available BAC and EST sequences as well as the synteny region with rice, several new markers were developed for the mapping. The *Scmv1* was finally assigned into two B73 BAC clones while *Scmv2* was assigned to a 300 kb region covered by a B73 contig consisting of three BAC clones. The allelic BAC clones from the resistant parent FAP1360A as well as two Chinese resistant lines for *Scmv1* were obtained. Correspondingly, a contig composed of three BAC clones from FAP1360A for *Scmv2* region was constructed and all the BAC clones were subjected to 454 sequencing. The remaining gaps were filled up by traditional sequencing. Ten and six candidate genes were discovered for *Scmv1* and *Scmv2*, respectively. Interestingly, none of the candidate genes appears to encode a homologue of known resistance genes. The candidate genes for both *Scmv1* and *Scmv2* are being validated by RNAi and complementary test. Isolation and characterization of the two resistance genes will lead to the discovery of novel classes of R genes with unique virus-targeting mechanisms.

P171

Maize transcriptome analysis upon *Fusarium* infection in relation with host and pathogen genotypes

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We investigated global gene expression in maize ears at several time points after infection with *Fusarium verticillioides*. In kernels at 48 h post infection with a fumonisin-producing strain, about 800 differentially expressed sequences were identified and nearly 10% assigned to the category cell rescue, defence and virulence. The expression analysis was extended to early (12, 24 h) and late (72, 96 h) phases after infection with a fumonisin-nonproducing strain. The mutant strain was able to activate host defence genes later than the wild type strain. When resistant and susceptible maize genotypes were compared, in the resistant lines the expression of defence genes were induced upon infection, indicative of a basal defence response against the fungus. In the susceptible genotypes defence genes were induced specifically after pathogen infection. The basal defence response was also active against several fungal species invading maize kernels.

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P172

Mapping and Functional Analysis of Brown-midrib2 (*bm2*) via RNA-seq

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Brown-midrib (*bm*) mutants are characterized by a reddish-brown color on their leaf midribs. This mutant phenotype is associated with reductions in lignin concentration and alterations in lignin composition. Biomass with reduced lignin concentrations is more easily digested by ruminants and is a more efficient feedstock for biofuel production. Therefore, analysis of *bm* genes can enhance our understanding of the biochemical pathways that lead to lignin accumulation, offering the promise of designing crops having reduced lignin concentration. At least five maize *bm* mutants have been identified, but only two of these (*bm1* and *bm3*) have been cloned. Here, we report the mapping and functional analysis of the *bm2* gene. The *bm2* gene was mapped to a 3Mb on chromosome 1 using a newly developed adaptation of BSA (Bulk Segregation Analysis) that exploits the power of next generation sequencing (Liu *et al*., in preparation). Analysis of RNA-seq data indicates that the *bm2* mutation alters the accumulation of transcripts from genes in the lignin biosynthetic pathway.

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P173

MiRNA-mediated auxin signaling involved in initiation and elongation of crown roots of maize under submergence condition

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Ten small RNA libraries from crown roots of maize seedlings under control and submergence of 1- 4 d and four degradome libraries from control and pooled submergence-treated roots of tolerant inbred (HZ32) and sensitive inbred (Mo17) were sequenced, respectively. In ten small libraries, a total of 187 unique sequ-seqs with high copy number were identified. In these sequences, 94 known zma-mRNAs and zma-miRNA*s were classified into 25 families, and 14 predicted new miRNAs were involved with 7 families. In addition, 78 were predicted as candidates of zma-miRNA. Comparing normalized reads of miRNAs in the submerged libraries with that of corresponding control, a set of differentially expressed miRNAs were identified in two inbreds, 83 in Mo17 and 77 in Hz32, respectively. 54 common miRNAs and miRNA*s were differentially expressed in two inbreds, and 23 and 29 responsive small RNAs were specifically detected in Hz32 and Mo17, respectively. In all, the majority of miRNAs was down-regulated, and a minority of miRNAs was up-regulated in both inbreds under submergence condition. The targets of differentially expressed miRNAs were identified by degradome sequencing, The genes in auxin signaling (ARF, TIR1) and genes in downstream of auxin signaling pathway (NAC domain encoding gene, ids1, sid1) were detected in degradome libraries. Potential miRNA targets were validated by 5′-RLM-RACE and real-time PCR. We suggested that miRNA-mediated auxin signaling potentially involved in initiation and elongation of crown roots of maize under submergence.

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P174

Molecular Cloning of *Opaque7*

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Maize has a large class of seed mutants with non-vitreous endosperms. This phenotype appears to be linked to the improper formation of protein bodies (PBs), where the major storage proteins - the zeins - are deposited. Although transposon tagging and positional cloning have been successfully used to isolate a number of genes specifying the normal vitreous phenotype, it has been far more difficult to clone *Opaque 7 (O7)*. There are two possible reasons for this: penetrance and lack of an active transposon nearby. The reference *o7-ref* mutant arose spontaneously in a W22 inbred line but appears to be less penetrant when introgressed into other lines. Given this variable penetrance, it might be difficult to identify the gene just by positional cloning. We, therefore, created new alleles by transposon tagging in a W22 background. The *mR^{nj}(Ac)* allele, about 25 cM proximal to *o7*, was used as reporter system to screen 900,000 seeds for *opaque* phenotype in a *mR^{nj}(Ac)/r; O7/O7* x *r/r; o7-ref/o7-ref* cross. Although several putative opaque mutants were isolated, only two were heritable. One of them had a 2-kb insertion at codon 496 of a 528-codon long acyl-CoA synthetase-like gene (ACS) that resembles the single *Ds* component of *double-Ds*, McClintock's original *Dissociation* element. The *o7-ref* allele is characterized by a 12-bp in-frame deletion (codons 350 to 353) in the second exon, proximal to the *Ds* insertion. The lack of mutants affecting other regions of the protein and the low recovery of mutants despite a large screen suggest possible pleiotropic functions. Although zein synthesis appeared to be unaltered in *o7-ref* and protein accumulation changed only slightly, PBs exhibited striking membrane invaginations. A plausible model consistent with these observations is that the ACS enzyme plays a key role in membrane biogenesis and that the altered PBs make the seed non-vitreous.

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P175

New mutants in accessible UniformMu seed stocks

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The UniformMu project is creating a public reverse genetics resource for maize that provides ready access to knockout mutations in thousands of maize genes. Thus far, over 24,000 unique germinal Mu insertions have been mapped in a set of 5,400 UniformMu F3 stocks using NexGen sequencing methods. We expect to map up to 40,000 insertions in 8,000 F3 lines by Summer 2011. The precisely mapped, germinal insertions are searchable online at MaizeGDB by BLAST and genome browser tools. This site also links users directly to high-quality, sustainable seed stocks distributed without charge by the Maize Genetics Stock Center. Annotation of insertions using a filtered AGP v2 gene set indicates that at least 79% of the Mu insertions are in genes (64%) or promoters (500 bp, 15%). The collection contains hits in at least 12,000 maize genes including 4,300 genes that have two or more insertion alleles. Multiple insertion alleles are especially valuable to researchers for confirming gene functions. Mu insertion frequencies genome-wide were highly correlated with gene density (R^2= 0.9); whereas the correlation with recombination frequency was lower by comparison (R^2 = 0.65). Statistical analysis of insertion clustering in the genome indicates that Mu preferentially targets compact regions (<1 kpb) that frequently are associated with 5'-ends of genes. A mixed Poisson model of Mu insertion frequency predicts two classes of Mu targets that differ by 4-fold in average frequency of Mu insertions indicating absence of strong "hotspots" in UniformMu. Features that distinguish the low and high frequency classes of insertion targets are under investigation. Our results confirm that Mu is a highly effective mutagen for comprehensive functional analysis of the maize genome. The UniformMu resource has increased 10-fold in the last year. Check now for new Mu inserts in genes of interest.

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P176

Nitrogen influenced transcriptome and metabolome analyses in maize

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Even though physiology of nitrogen (N) metabolism is one of the extensively studied metabolic components in plants, information on nitrogen influenced transcriptome in maize is limited. Two profiling technologies namely microarrays and next-generation-sequencing (NGS) were used to study the tissue, genotype, development and nitrogen treatment specific mRNA accumulations. While relatively older technology, microarrays, provided nearly 6 million data points for pairwise comparison of selected parameters in 17 experiments, NGS provided nearly 200 million data points within one experiment that contained 24 samples indicating improved coverage provided by NGS. Microarrays provided valuable information about genes whose expression was markedly altered by variations in nitrogen availability or as a function of genetic ability. Approximately 30,000 Maize genes were represented in the microarray experiments, of which nearly 16,000 exhibited differential expressions in response to N. The identified differentially accumulating transcriptome illustrated the complexity of environmental impact on nitrogen utilization efficiency. Nitrogen influenced transcriptome was delineated from parameters such as tissue and genetic specificity. Genes that selectively expressed in developing ear shoot were identified. This data set was compared with a simultaneous profiling experiment performed using the cultured kernels sampled at different stages of the ear development. Three major concepts namely, N sufficiency, N deficiency and N recovery were studied in the kernels grown in controlled environment with variable supply of nitrogen to the culture. Growing kernel weights and free amino acid profiles showed expected readings in kernels and cobs. Genes responding to variable supply of nitrogen in growing kernels were identified.

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P177

Remobilizing Ds transposons for targeted mutagenesis in maize

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A sequence-indexed collection of over 1,700 *Ds* insertion lines in maize was generated as a genetic resource to facilitate gene function discovery and characterization studies. To validate the feasibility of using these mapped *Ds* insertions as launchpads for local mutagenesis, we targeted several loci whose gene functions are traceable in the kernel. These experiments aim to identify optimal genetic and/or physical distance between the donor *Ds* (d*Ds*) and target loci and to develop estimated population size requirements for recovering multiple insertions. Target loci included *bz2*, located on chromosome 1L, *sh1* and *bz1* on chromosome 9S and the *a1* locus on chromosome 3L. Several d*Ds* lines for each target gene were selected by searching the mapped d*Ds* insertion database at PlantGDB (http://www.plantgdb.org/prj/AcDsTagging), The exception was *bz2*; there was a single d*Ds* present in that region, estimated to be within 0.1 cM (31 kb) of the target. A similar approach was taken for the other target genes, except that the d*Ds* lines selected were estimated to be in the range of 0.5- to 25 cM from target loci. Sequence analysis of the target loci in 19 *sh1*, 7 *bz1* and 11 *a1* individuals yielded 6, 4 and 6 new *Ds*-derived alleles in *sh1*, *bz1*and *a1*, respectively. For *bz2*, 5 new *Ds*-derived alleles were recovered. Mutagenic efficiency of the different d*Ds* lines as a function of genetic and physical distance, and the locations of the *Ds* insertion alleles will be presented. Implications of these results on the putative population sizes and strategies for using this collection of d*Ds* lines as a reverse genetics tool will be discussed and we will present our preliminary results of a reverse genetic screen to identify *Ds* insertion in the *PPdK1* gene, necessary for C4 photosynthesis.

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P178

Repeat-associated small RNAs in the shoot apex vary among parents and following hybridization in maize

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Maize is characterized by high degrees of genomic complexity, genetic diversity, and hybrid vigor. Variation in small RNAs (sRNAs) influences both genome structure and expression, and thus may contribute to hybrid vigor. Maize hybrids produce larger and greater number of organs compared to their inbred parents, indicating differences in meristem activity are a key contributor to heterosis. We used Illumina deep sequencing to assess how sRNA populations within the seedling shoot apex vary between two maize inbred lines (B73, Mo17) and their reciprocal hybrids that exhibit high heterosis. Most classes of sRNAs do not differ in relative abundance between the parents or between parents and hybrids, the major exceptions being sRNAs associated with the amplification of post-transcriptional gene silencing. These sRNAs include non-canonical sRNAs, 21-nucleotide small interfering RNAs (siRNAs) produced from variant alleles at *TAS3* loci, and 22-nt siRNA clusters derived from retrotransposons. Hybridization combines these parental differences in an additive fashion with a trend toward dominance, resulting in hybrids that differ from both parents in their complexity of sRNAs. Loss of the RNA-dependent RNA polymerase (RDR2) encoded by the *mediator of paramutation* (*mop1*) locus does not suppress heterosis for B73 x Mo17, supporting our finding that nearly all 24-nt siRNA clusters are found in both inbred parents and do not change upon hybridization. Our results suggest that hybridization increases the RDR2-independent amplification of post-transcriptional silencing in the maize shoot meristem, which is associated with more effective silencing of retrotransposon sequences and greater growth. The changes in RNA silencing of repeat sequences are intriguing with respect to the properties of hybrid vigor in maize, as they differ between parents, their effects are distributed throughout the genome, and their potential for amplification could generate non-additive phenotypic effects.

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P179

Reshaping of the maize transcriptome by domestication

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Several studies have documented the genomic changes that have accompanied the domestication of maize from teosinte. We sought to characterize how the transcriptome has been altered by domestication. Expression profiling was performed on 38 diverse maize genotypes and 23 teosinte genotypes. The probesets were filtered using comparative genomic hybridization to remove probes that detect polymorphic DNA sequences. The remaining probes were used to estimate gene expression levels in the maize and teosinte genotypes. We identified 612 genes with altered expression levels in maize relative to teosinte and another 370 genes with altered expression in out-crossed teosinte individuals relative to inbred genotypes. Co-expression networks were built separately for both maize and teosinte genotypes. There was evidence for substantial differences in the expression conservation for many genes and many co-expression networks were altered in maize relative to teosinte. A number of the previously identified domestication-related genes were found to exhibit differential expression or be present in co-expression networks that have been re-wired in maize. This analysis helps to identify potential targets of domestication and to further understand the physiological changes that have accompanied domestication.

Funding acknowledgement: National Science Foundation (NSF)

P180

SNP-based Bulked Segregant Analysis for the Speedy and Economical Mapping of Maize Mutants

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A wide variety of approaches are available for mapping maize mutants. Unfortunately, most of these approaches are frustratingly slow and/or expensive. Iowa State University's Genomic Technologies Facility (GTF) now offers a fast and economical mutant mapping service based on the approach of Liu et al., 2010, Genetics 184:19-26. A set of 1060 validated SNP markers that are distributed across the whole genome were grouped into 30 plexes. These markers are assayed using the Sequenom iPlex genotyping system. The quantitative nature of Sequenom-based SNP assays enables a time- and cost-efficient strategy to genetically map mutants via quantitative bulked segregant analysis (BSA). Allelic ratios of SNP assays are compared between pools of mutants and wild-type sibs. SNP markers that are linked to the mutant exhibit different allelic ratios between mutant and wild-type pools. Over the last two years, the GTF has used this system to genetically map >100 maize mutants for maize researchers around the world.

P181

Sequencing the maize methylome

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We have sequenced the methylome of B73 and Mo17 inbreds at 28.7 and 19.7 fold coverage, respectively, using bisulphite conversion and Illumina next generation sequencing. In agreement with previous results, we have found that the vast majority of cytosine methylation in all three contexts (CG, CXG, and CHH) is found in intergenic regions and transposable elements, with exons containing only 5-10% CG methylation. CHH methylation is rare and confined mostly to intergenic regions. Analysis of cytosine methylation in promoters, first and last exons, splice and transcription start sites indicates methylation may be guided by regulatory signals. Phasing and asymmetry of cytosine methylation in each context will be assessed. Exemplars of transposable elements that differ in DNA methylation will be presented, along with comparison with existing RNA-seq, small RNA and chromatin immunoprecipitation datasets. Extensive variation between Mo17 and B73 was detected in regions of primary sequence similarity. Strategies for determining the inheritance and biological significance of epigenetic variation will be discussed.

P182

Structural and metabolic transitions of C3 rice and C4 maize leaf development and differentiation determined by a systems biology approach

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Compared to C3 grasses, C4 grasses achieve a higher photosynthetic efficiency through biochemical and structural adaptations. This study aims at comparing development and differentiation of the rice C3 leaf to the maize C4 leaf, to better understand the C4 specialization. A developing maize leaf is characterized by undifferentiated, non-photosynthetic sink cells at the base and highly specialized mesophyll (MCs) and bundle sheath cells (BSCs) at the tip. The profiles of proteomes of the maize leaf development gradient, as well as isolated vascular bundle with surrounding BSCs, were determined using large scale mass spectrometry, complemented with qualitative and quantitative microscopy analysis. Protein accumulation profiles and hierarchical cluster analysis determined the kinetics of organelle biogenesis, cellular structure formation, metabolism and co-expression patterns of proteins in important functional pathways. Two main expression clusters containing several subclusters were observed, suggesting that a limited number of regulatory networks organize concerted protein accumulation along the leaf gradient. Co-expression with BSC and MC markers provided strong candidates for further analysis of C4 specialization. We describe five developmental maize leaf transitions which provide a conceptual and practical template for further analysis. A similar strategy was applied to developing rice leaf; here we will report the preliminary integrated large scale microscopy and proteome analysis of such rice leaf, which will facilitate comparison of C3 and C4 photosynthesis and the underlying regulatory networks.

Funding acknowledgement: National Science Foundation (NSF)

P183

The Genetics of Sustainable Agriculture: Does Maize Gene Expression Respond to Soil Management?

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Environmental issues associated with conventional agricultural systems include soil erosion, water and air pollution, and reduced natural biodiversity. Developing economic sustainable agricultural systems could lead to a solution to these issues. This project relates the effects of using sustainable and conventional management practices on plant and soil health to gene expression in maize. Sustainable and conventional soil management practices expose maize plants to different growing conditions such as level of soil moisture retention, source and amount of available nitrogen, and root-zone temperature. It is expected that expression levels of candidate genes will change between treatments due to changes in plant and soil properties. Gene expression in leaf tissue will be analyzed using quantitative real-time PCR. The genes to be studied are involved in carbon and nitrogen metabolism, defense against pests and disease, senescence, and tolerance to environmental stress. Protein expression levels will be analyzed using Western hybridization. Results of this study will compare differences in plant and soil properties between treatments to differences in expression levels of candidate genes and proteins. This research will help scientists and farmers better understand the effects of well characterized sustainable agricultural practices from a new perspective with a molecular emphasis. The results of this study could provide incentive for farmers to implement environmentally sustainable techniques and for scholars and extension agents to further promote their use. The findings from this study could also provide important information for plant breeders who wish to develop maize plants that excel under conditions experienced in sustainable management systems.

Funding acknowledgement: Environmental Protection Agency (EPA)

P184

The Grass Transcription Factor ORFeome (TFome) Project

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The control of gene expression is central to all cellular processes. Transcription factors (TFs) function in networks, in which a TF may control the expression of another, which in turn may modulate the expression of additional downstream TFs. An emerging theme is the identification of these regulatory networks in which TFs participate. It is estimated that the number of TFs in maize is about 7% of the genome, representing more than 3500. As part of a long-term effort to investigate and understand grass regulatory networks, we have initiated The Grass Transcription Factor ORFeome Project (TFome). We have already cloned more than almost 200 transcription factors from maize and more than 75 from rice, with many more in the pipeline. Full-length ORFs or cDNAs (flcDNAs) for TFs are being identified and then cloned into Gateway® Entry vectors that will permit the facile recombination into plasmids for expression in plants or microorganisms. Many of these clones have also been recombined into destination (pDest) vectors suitable for overexpression and protein production. Clones for these TFs are being made publicly available to researchers through Addgene (www.addgene.com). Information on available clones is being posted at the GRASSIUS (www.grassius.org) web resource. As part of the database development we have proposed a set of rules for naming TF proteins in the grasses Plant Physiology 2009 149(1) p4-6. We are also conducting a survey of the entire TF repertoire in the maize genome and will report on our findings. In the long term it is envisaged that the a complete maize TFome will be generated which will be immensely useful in conducting yeast two-hybrid and one-hybrid studies aimed at finding TFs and their partners for any gene of interest. This project was funded by grant NSF DBI-0701405.

Funding acknowledgement: National Science Foundation (NSF), Ohio Plant Biotechnology Consortium (OPBC)

P185

The degree of ascertainment bias in SSRs, SNPs and genotyping-by-sequencing (GBS)

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In the recent past, chip-based high throughput genotyping has facilitated genome-wide studies in many crops including maize. However, single nucleotide polymorphism (SNP) chip data suffer from ascertainment biases caused by the SNP discovery procedure wherein ascertainment panels consisting of small numbers of individuals from selected populations are used. The consequences of ascertainment bias are biased estimates of recombination, genetic variability and population structure. These biases can be severe, depending on the makeup of the panel.

Genotyping-by-sequencing (GBS) is a more cost effective and open platform than either SNP arrays or SSRs. In maize, high throughput next generation sequencing of regions flanking restriction sites enables the detection of millions of tags in a single reaction, many of which will be polymorphic and Mendelian. GBS has the potential to detect novel and rare alleles, and hopefully will exhibit less ascertainment bias than other approaches. We have generated GBS genotyping data for a diverse maize association panel comprised of 282 lines that previously has been genotyped with the maize 55K Illumina SNP array and with SSR markers. Here we will compare inferences from GBS with those from the 55K SNPs and SSR markers. Our characterization of the ascertainment biases in data sets from the different marker types may help to correct conclusions drawn from previous studies. Accurate inferences of relatedness are needed to estimate population structure, conduct GWAS experiments, and make genomic selection breeding value estimates.

P186

The inheritance of small RNA expression patterns in Arabidopsis hybrids

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Heterosis, also known as hybrid vigor, refers to the phenomenon wherein an F1 hybrid produced from crossing two cultivars of the same species or two different species displays superior phenotypes compared to the inbred parents. Regardless of its practical application and scientific importance, the molecular mechanism underlying heterosis is not completely understood. In recent decades, knowledge of the regulatory roles of small RNAs has greatly improved our understanding of many basic biological questions. We hypothesized that small RNA could be involved in the molecular basis of heterosis, since small RNA and the epigenetic inheritance patterns it controls fit with many of the inheritance characteristics of heterosis. We therefore applied a global small RNA profiling approach using Illumina sequencing to characterize the inheritance of small RNA expression patterns in Arabidopsis. Two *Arabidopsis thaliana* accessions, *Columbia* and *Landsberg erecta*, were crossed reciprocally to produce hybrids. These hybrids show heterosis, as characterized by leaf area and plant size. The small RNA expression patterns of both parents and two hybrids were compared. We report that the distinguishing expression pattern of small RNAs in hybrid Arabidopsis is non-additive inheritance of the low-parent pattern (~50%), followed by additive expression (~36%). Analysis of the genomic origin of non-additively expressed small RNAs suggested that they are mostly heterochromatic siRNA associated with maintaining genome stability. Down-regulation of heterochromatic siRNA thus appears to occur in Arabidopsis F1 hybrids and may play a role in the increased growth and viability of these plants.

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P187

The role of *Pap* and *Pho1* genes in the maize phosphate starvation response

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Phosphate is an essential macronutrient required for plant growth and development. A number of genes have been previously identified in *Arabidopsis* to play a key role in plant phosphate regulation and responses to phosphate starvation. Now, with the availability of the B73 maize genome sequence, we are beginning to translate this information from *Arabidopsis* to maize. Here, we report initial identification of maize homologues of the genes At*Pap26*, encoding a major acid phosphatase activity implicated in phosphate mobilization and scavenging, and At*Pho1*, involved in phosphate loading into the xylem and phosphate sensing. We have identified four maize genes that are close homologues of At*Pap26*, and four further genes that are homologous to At*Pho1*. In a preliminary analysis, we have observed differential expression of maize *Pap26* –like and *Pho1*-like genes between the roots and leaves of young seedlings, indicative of sub-functionalization among gene paralogs. To test orthology relationships between *Arabidopsis* and maize, we are performing heterologous complementation experiments, expressing maize coding sequence under the control of *Arabidopsis* promoters, in *Arabidopsis pap26* and *pho1* mutants. To further test the functionality of maize *Pho1* homologues, we have initiated an *Ac/Ds* transposon tagging effort, targeting two of the four genes.

Funding acknowledgement: National Science Foundation (NSF), LANGEBIO-CINVESTAV. Mexico

P188

The subfunctionalization of the maize phytochromes *PhyB1* and *PhyB2* is background-specific

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Mu insertion alleles *phyB1* and *phyB2* were introgressed in the B73 and W22 inbred backgrounds. B73 and W22 *phyB* mutant series (which included the wild-type segregant, the two single mutants and the double mutant) were phenotyped at maturity for ten morphological traits. Each inbred background revealed distinct subfunctionalization patterns between the two *PhyB* paralogs. For the majority of the traits measured, the *phyB1 phyB2* double mutants were significantly different from the wild-type segregant, while the single *phyB1* and *phyB2* mutants showed additive or dominant roles for each paralog. Collectively, these results suggest the presence of background-specific genetic modifiers acting on the phytochrome signal transduction pathway. In addition, considerations regarding the influence of low red to far-red light ratio at dusk and the shade avoidance syndrome on the topology of a maize stand will be presented.

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P189

Transcriptional and metabolic changes during induced senescence in maize

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Senescence is a highly regulated process that leads to death of a cell, an organ, or an organism. In plants, leaf senescence is characterized by catabolism of proteins, lipids, and chlorophyll followed by remobilization of breakdown products to the sink. Since, onset of senescence results in cessation of photosynthetic productivity, delayed senescence can potentially extend carbon fixation and increase grain and biomass yield. However, biochemical and molecular mechanisms regulating leaf senescence are not well understood. In maize, pre-mature leaf senescence can be induced by the absence of a seed sink. We induced leaf senescence in B73 inbred of maize by preventing pollination and assessed differential metabolic and global transcriptional changes in leaves and internodes at six stages during normal grain filling. Early senescence was associated with increased accumulation of glucose in leaves, and to a lesser extent in internodes during the early and middle period of grain filling. Interestingly, these differences were not observed at later stages, likely due to loss of photosynthetic activity in early-senescing plants. In contrast, pentose sugars showed lower accumulation in leaves and internodes of early senescing plants. We used a NimbleGen microarray to study the global expression changes upon induced senescence. Substantial transcriptional reprogramming in leaves was observed at 18 DAP (days after pollination) that is consistent with major changes in the metabolic profile. We observed early induction of genes involved in leaf senescence-related processes including program cell death, chlorophyll breakdown, and β-oxidation of fatty acids. Expression of genes involved in sugar transport also showed trends consistent with accumulation of these metabolites in leaves and internodes. We are now working on identification of novel genes that will be potential candidates involved in the regulation of senescence. Transcriptional changes in the context of accumulation and transportation of metabolites including storage/structural carbohydrates will be discussed.

Funding acknowledgement: Department of Energy (DOE)

P190

Transcriptome Analysis and Functional Annotation of Nitrogen-Related Genes in the Maize B73 Genome

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Nitrogen (N) plays a key role in plant growth and development. Therefore, knowledge of the genomic location and function of genes controlling the metabolisms surrounding N use efficiency (NUE) are very important to its improvement in maize. Focusing on 38 previously annotated genes known to have involvement in nitrogen, carbon, and energy metabolism, protein sequences were extracted, and over 3000 responding ESTs were identified from 2 million maize ESTs deposited in NCBI. Subsequently, the original 38 protein sequences were combined with these 3000 ESTs and used as queries to search the fully sequenced maize B73 genome. One hundred and ninety six putative maize N-related homologous gene regions were defined and annotated. RNA profiling data confirmed previous hypotheses surrounding the activity of these genes under varying N treatments in maize B73 cultivars. Further analysis of the data may reveal previously unknown gene expression responses to N supply. Transcriptome analysis and annotation of selected N-related genes in the maize genome provide a valuable source to confirming and revealing gene activity as well as understanding gene distribution and characteristics. Through utilization of the newly sequenced B73 genome in conjunction with data from previous studies, efficiency and overall success in improvement of N use efficiency in maize can be achieved. Moreover, methods utilized here could be applied to other crops and traits in the future.

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

P191

Variation for allelic gene expression in maize

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Changes in gene expression play a major role in the adaptive evolution that precedes and accompanies speciation. The relative contributions and interplay of *cis*- and *trans*- acting regulatory factors to this evolutionary process in plants, however, remain poorly understood. To investigate these issues, we are describing and fine mapping the cis-acting genetic components involved in gene expression variation in the *Bz1-Sh1* region of maize. The *Bz1-Sh1* region was chosen because it is highly dynamic among maize inbred lines, consisting of multiple transposon insertions and deletions, rearrangements, and a high propensity toward recombination. This naturally occurring intergenic polymorphism provides a potential substrate for expression effects on neighboring genes. To explore the relationship between genomic context and gene expression, we are using 454 sequencing technology to quantify the *cis*-effects on heteroallelic gene expression variation of eight of the thirteen genes in the *Bz1-Sh1* region in several maize hybrids. Initial analyses indicate that most of the genes in the *Bz1-Sh1* region display striking variation in expression patterns among parental genotypes in the same genetic background and across different tissue types, despite high levels of sequence conservation at the nucleotide level among protein-coding sequences.

P192

slm1 (suppressor of lesion mimic-1) encodes an NBS-LRR protein required for the expression of the lesion mimic mutation les23 in maize (Zea mays L.)

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Maize disease lesion mimic mutants provide an excellent model to study the genetic mechanism of cell death in plants. The phenotype of many lesion mimics varies in different genetic backgrounds. A previous study identified a major quantitative trait loci (designated as slm1) on chromosome 2 in the maize inbred line Mo20W suppressing the expression of a recessive lesion mimic mutation, les23. Fine mapping using a BC8F2 population has narrowed down slm1 to a 77.6 Kb region containing a single candidate gene for slm1. The full-length coding region of slm1 was cloned from Va35, the background in which les23 was originally discovered. The sequence of slm1 predicts that it encodes an NBS-LRR protein of 1066 amino acids. The full-length slm1 CDS has also been cloned from Mo20W and it contains a 2 bp insertion that brings a stop codon in frame and causing the gene to encode a truncated protein of 332aa. Our data suggest that a functional NBS-LRR gene is required for the cell death phenotype underlying the les23 mutation while the presumed loss-of-function of this gene in Mo20W may negatively affect (suppress) the lesion phenotype in a dosage-dependent mode (partially dominant). Our further characterization of slm1 and the cloning of les23 will promise a better understanding of the nature of cell death pathway in plants.

Funding acknowledgement: National Science Foundation (NSF)

P193

Establishing in vivo maize doubled haploid production at the University of Illinois for education and research purposes

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The application of doubled haploid production is largely used in the private sector to reduce the length of breeding cycle through creation of 'instant inbreds'. However, few academic institutions have doubled haploid production capacity. By establishing dihaploid capacity, students can be familiarized with the new industrial technology in modern germplasm improvement through hands-on instruction and be more prepared for industry careers. Furthermore, we desire to offer doubled haploid production capacity as a service to our faculty in corn improvement as well as researchers at other public sector institutions, as it saves not only time in line development, but resources as well.
The introduction of inducer lines and artificial chromosome doubling using colchicine has enabled mass production of maize doubled haploid, with the realized chromosome doubling rate at 20-30%. We seek to improve the process both in terms of efficiency and ease-of-use by 1) estimating haploid induction rate of different genotypes, 2) applying environmentally-friendly colchicine alternatives, 3) establishing early indication method for differentiating doubled haploid from haploid plants. The inducer line, UH400 from University of Hohenheim, was used for creating haploid seeds, and color marker system was used for separating haploids. Five European corn borer resistance and 66 Ex-PVP F1 populations were used as donor materials representing various categories of genotypes. Although the fertility of anthers is one means to determine the successfulness of the doubling treatment, early screening for the doubled haploids would help save the growing space and time in large scale doubled haploid production. Among the possible methods, stomata length measurement on leaf is easy and cheap. Moreover, colchicine is toxic to the environment and to the user, and the use of anti-microtubule herbicides as doubling reagents would be more environmentally-friendly alternative method to the use of colchicine.
Preliminary results on stomata length measurements showed on average there was significant stomata length difference between haploid and doubled haploid leaf. When the length was compared at each leaf stage between haploid and doubled haploid, the largest difference was found at the early 1-2 leaf stage. This result is encouraging for selecting doubled haploid from haploid at early stage. More results on induction rates on different donor genotypes, chromosome doubling using anti-microtubule herbicides and stomata length measurements will be presented.

Funding acknowledgement: USDA National Institute of Food and Agriculture, Agriculture and Food Research Initiative

P194

Exploiting the extraordinary transposable element-mediated diversity of maize to illustrate key genetic concepts to undergraduates and high school students

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Our Dynamic Genome courses use transposable elements (TEs) to illustrate key biological concepts through authentic research projects. Funded by an HHMI Professors grant, these courses replicate the Wessler research laboratory as a classroom where projects are derived from ongoing research studies. In a specially designed facility, students move from a computer classroom where they use bioinformatics to design and test hypotheses to the wet laboratory where they generate data that is often submitted to existing databases. This poster summarizes a class module that demonstrates the extraordinary level of TE-mediated genome variation in maize and the synergy between the research and classroom laboratories. The module began in the research laboratory where a graduate student working on the Maize Genome Annotation Project used a computational approach to identify nonautonomous TEs in the B73 reference genome. Much like bingo, each student then selected the name of one of the TEs. From here they used genome browsers to identify intronic insertions, designed PCR primers to flanking exons, amplified the region from maize DNAs (that they had previously extracted from a panel of inbred lines) and resolved the products on gels. Although these loci had never been tested previously, the extraordinary level of maize genomic diversity virtually guaranteed that some insertion site polymorphism would be detected. This module has proven to be extremely effective at teaching college freshman and high school juniors complex genetic concepts such as gene structure, genome organization, and evolutionary principals. In addition, students learn key research skills including bioinformatics, experimental design, and data collection and analysis.

Funding acknowledgement: Howard Hughes Medical Institute and the National Science Foundation

P195

Maize: Mysteries of an Ancient Grain – a Traveling Museum Exhibition

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Explore the science of how maize has evolved to become one of the most significant crops to humankind for thousands of years and why it continues to surprise us. Schedule requests are now being accepted from science, agriculture and natural history museums for the new traveling exhibition, "Maize: Mysteries of an Ancient Grain". The content of this visually stunning and educationally rich exhibition focuses on evolution and genetic diversity through the study of maize.

This is a collaboration between the multi-institutional project "Genetic Architecture of Maize and Teosinte" (http://www.panzea.org/) (NSF Award #0820619) and the Paleontological Research Institution and its Museum of the Earth (http://www.museumoftheearth.org/). Interspersed with "Current Science" callouts, themes include how ancient civilizations domesticated maize from its ancestor teosinte; genetic diversity, inheritance, and evolution; the challenges of today's crop improvement; and the work of seed conservation projects. In addition to large walk-around panels the exhibit includes freestanding activity desks with related interactive lessons, and "Why I Became a Scientist" set of biographical stories of a diverse group of scientists.

Exhibition includes:
• 2 easy to install 15' wide x 7'-6" tall pop-up "S" display panels that are two-sided and come with travel cases
• 5 interactive free-standing desks that click together and come in travel cases Dimensions: 48" wide x 24" deep x 32-36" tall and are ADA accessible
• 8 wall mounted panels: 18" x 24" for project credits and scientist
• templates for ads and collateral

Venues: Science, agriculture, natural history museums, libraries, universities and community centers. Limited funding may be available, call for details.

If interested in having the exhibition come to your venue, please contact

Museum of the Earth:
Cathy Blackburn, Director of Exhibitions: blackburn@museumoftheearth.org or
Beth Stricker, Exhibitions Manager: stricker@museumoftheearth.org

For curatorial or educational content questions please contact Theresa Fulton: tf12@cornell.edu

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P196

MaizeResearch.org: Outreach for the maize genetics community

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At the 52nd Maize Meeting, the Maize Genetics Executive Committee called for improved public communication of research relevance and results (press releases, etc.) from the community at large. They argued there was a need to communicate basic and applied research in a way that is accessible and comprehensible to non-scientists. In pursuit of this goal, MaizeResearch.org was developed by Anastasia Bodnar with technical assistance from Carson Andorf. This website is designed to be an information hub for all things maize research. To our knowledge, this is the only broad initiative to discuss maize research on the web. The blog format allows scientists to interact with each other and with non-scientists in unique ways. The website promotes the work of maize researchers, provides a venue for communication between scientists, and makes the research more accessible to non-scientists. Scientists can use MaizeResearch.org to engage an audience beyond the scientific journals. We encourage others to participate in this outreach project. There are many opportunities, including providing summaries of published research, writing articles about research or current events, searching the web for interesting maize related stories, and much more. Please email Anastasia Bodnar at maizeresearch1@gmail.com for details.

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P197

UC Davis Plant Breeding Academy

(submitted by Rale Gjuric <rgjuric@ucdavis.edu>)
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With over 100 years of history of plant breeding and diversity of crop that ranges across vegetables, fruits, nuts, grains, forages, ornamentals and turf, University of California Davis is one of the world's leading institution in plant breeding and plant breeding education.
The Plant Breeding Academy is a two-year course designed to address the reduced numbers of plant breeders being trained in academic programs. The course will develop the skills and abilities of the participants in practical plant breeding, genetics and statistics to enable them to become independent breeders. It provides specific, practical information as well as the theoretical basis for designing and managing successful plant breeding programs. The course is modeled after an MBA, such that participants can retain their jobs while attending the program. Participants are in class for six 6-day sessions over 2 years. The program has gained very broad recognition attracting large number of participants from 39 seed companies and 17 different countries.

P198

Using the maize TFome project to *F*oster the *I*ntegration of *R*esearch with Undergraduate *E*ducation (FIRE)

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There is a growing recognition that in order to promote careers in the sciences it is important to let students have an opportunity to perform research during their undergraduate careers. Whereas this goal is widely recognized it is difficult to implement by letting students experiment in traditional research laboratories simply because of the numbers involved. At the University of Toledo we have initiated a program to *F* oster the *I* ntegration of *R* esearch with undergraduate *E* ducation (F.I.R.E.). The first implementation of this is to incorporate aspects of an NSF PGRP project into an existing Molecular Genetics Laboratory taken mostly by second and third year undergraduates. As part of the PGRP project to investigate and understand grass regulatory networks, we had initiated The Grass Transcription Factor ORFeome Project (TFome). The long term goal is to generate a complete maize TFome (~3500 TFs) that will be immensely useful in studies aimed at finding TFs and their partners for any gene of interest. This type of project lends itself very well to incorporation into undergraduate education as the collection is built up over several years. Here we report on a teaching module that was developed and integrated into a class and can be readily adopted into similar classes at other institutions. In this class student pairs were each assigned to identify and clone a novel transcription factor from corn in the "Keys of Corn Project". A set of five laboratories were designed that introduced students to database mining, the polymerase chain reaction, gene cloning, bacterial transformation, and the use of bioinformatics to characterize a novel gene. To date over 250 students have participated in this project and cloned over 100 TFs while learning database mining and gene cloning skills.
This project was funded by grant NSF DBI-0701405.

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P199

A genome-wide association study of 1495 transposable elements in Zea mays

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Transposable elements (TEs) have an important role in the structure and evolution of plant nuclear genomes and may also contribute to phenotypic variation. More than 75% of the maize B73 genome is comprised of at least 1500 class I (retrotransposons) and II (DNA transposons) TE sub-families that are non-randomly dispersed at highly variable copy numbers (1 to >10,000 copies). Given such tremendous diversity for copy number variation among TE sub-families within a single genome, we postulate that heritable copy number variation exists within TE-subfamilies across multiple genomes and that the source of such genetic variation can be mapped to a genomic location. To that end, a genome-wide association study (GWAS) was conducted on standardized measures of Illumina read counts (reads per kilobase per million mapped reads; RPKM). A total of ten million HapMapv2 SNPs were tested on RPKM values of 1495 TE sub-families from 99 teosinte inbred and maize landraces and inbred lines. A difference in Illumina DNA library preparation was discovered to be the cause of clustered associations in the maize genome, which subsequent analysis accounted for in the statistical model. The process of modeling and association results will be presented and the implications of our findings will be discussed.

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P200

A genome-wide view of breeding history and selection in North American maize lines

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Since the advent of modern plant breeding in the 1930s, North American maize has undergone dramatic changes in its adaptation to high input agriculture. In spite of a strong genetic contribution to the historical increase in yield, little is known about the underlying genomic changes. We used the recently released Illumina 55k SNP genotyping array to characterize the genomes of a large number of historical North American landraces, early inbreds and more recent public and private maize lines. We thereby obtain a unique view of the genetic structure and composition of modern and historic breeding material and the genomic changes that have occurred over time. We apply a newly developed genome-scanning method to detect specific loci that are associated with recent selection in different genetic backgrounds.

Funding acknowledgement: United States Department of Agriculture (USDA)

P201

Bayesian-based GWAS: Identification of Loci Controlling Variation in Yield Component Traits in the Nested Association Mapping (NAM) Population

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Advances in next generation sequencing (NGS) technologies and the development of Bayesian statistical methods enable the genome-wide dissection of the genetic control of phenotypic traits. Yield component trait data were collected from 4,892 RILs in the Nested Association Mapping (NAM) population. Genotypes of NAM RILs were imputed from maize Hapmap1 as well as a set of 0.94 million genic SNPs identified via analysis of RNA-seq data generated as part of the NSF-funded SAM project (M. Scanlon, PI). After SNP pruning, a Bayesian method was used to fit all the remaining SNPs simultaneously to estimate SNP effects. The estimated breeding values (EBV) computed with different methods and different SNP sets were evaluated and compared. SNP associated loci controlling yield component traits were detected, several of which are consistent with previously generated QTL results.

P202

Breeding Maize for Resistance to Pink Stem Borer (Sesamia nonagrioides Lefebvre)

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In Turkey, pink stem borer (Sesamia nonagrioides Lef.) is one of the primary species attacking maize (Zea mays L). Therefore diallel crosses were conducted to study the heritability and inheritance of pattern of resistance of maize inbred lines to pink stem borer.
In 2008-2010, 48 maize inbred lines resistance to pink stem borer were developed by ÇUTAEM. Based on the clustering of DNA analysis, level of resistance to pink stem borer and their ability to produce seeds, 7 maize inbred lines resistant to pink stem borer were selected from these 48 lines. In addition, two lines namely 'Frmo 17 and Frb73' susceptible to pink stem borer were also included. Totally 9 inbred were crossed in diallele manner, excluding reciprocal to determine their combining ability and heritability of resistance to pink stem borer. All 36 F1 hybrids along with 9 inbred lines were planted as second crop in 2010, according to randomized completely blocked design with four replications. 25 larvae of pink stem borer were inoculated to each of the plant at 10-12 leaves stage. The level of resistance of inbred lines to pink stem borers were determined by measuring the length of tunnel made by insects as described by Guthrie and his colleagues (1978). Inbred lines having tunnel length ranging between 0.0-2.0 cm were considered as most resistant lines, whereas lines 'FrB73 and FrMo 17' were considered as susceptible genotypes having tunnel length of 46.6 and 45.9 cm respectively. 7 out of 8 hybrids having ÇukKrt35 as a one of the parent, with tunnel length ranging between 0.0-2.0 cm were considered as most resistant lines, whereas, rest of the one hybrid was considered resistant line with tunnel length of 3.2 cm. All other hybrids were considered as susceptible to pink stem borer. The yield of the resistant inbred lines used in the diallele crosses varied between 301.7- 401.3 kg/da, whereas susceptible lines Frmo 17 and Frb73' have yield of 13.2 kg/da and 15.2 kg/da respectively. Similarly yield of resistant hybrids varied from 680.3 kg/da to 998.4 kg/da, while susceptible hybrids have yield ranging between 268.3-38.6 kg/da. Inbred line 'ÇukKrt35' could be used in maize breeding program for developing hybrids resistant to pink stem borer.

Funding acknowledgement: TUBİTAK

P203

Breeding Potential of Semi-dwarf Corn for Grain and Forage in the Northern U.S. Corn Belt

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Conventional, non-dwarf corn (*Zea mays* L.) hybrids typically grown in the northern U.S. Corn Belt are more than 2 m tall and have a 75-100 day relative maturity (RM). Compared to conventional hybrids, semi-dwarf corn cultivars have lower ear placement, higher ear-to-stover ratio, and lower grain moisture at harvest. COPOP1 is a semi-dwarf (1 m tall), open-pollinated population known to contain the *reduced plant 1* (*rd1*) gene and possibly other minor dwarfing genes. The objectives of our study were to assess COPOP1 for grain and forage production, estimate genetic variances and broad-sense heritability of COPOP1, and develop COPOP1 subpopulations that exhibit heterosis. We evaluated COPOP1 with four commercial hybrids at three plant population densities. Grain yield of the conventional hybrids was nearly double that of open-pollinated COPOP1. Grain moisture and forage quality traits, however, were found to be favorable in COPOP1. Testcross populations were developed by crossing COPOP1 to two divergent inbred testers, LH227 and LH295. Heritability was significant for grain moisture, plant height, and ear height in both testcross populations but was significant for grain yield only in the LH227 testcross population. We then evaluated bulk Cycle 0 and Cycle 1 testcross populations, nine semi-dwarf by semi-dwarf hybrids, COPOP1, and three commercial hybrids at two plant population densities. Grain yield was not different between Cycle 0 and Cycle 1 in either testcross population. None of the semi-dwarf hybrids had higher grain yields than COPOP1. Our results indicate that COPOP1 is a good source of useful variation for improvement of northern U.S. Corn Belt germplasm. Further selection, however, must be conducted to produce significant improvement in COPOP1 subpopulations.

Funding acknowledgement:

P204

Breeding Specialty Starch Maize Using Exotic Genetic Resources for Gene Discovery of Novel Alleles and Modifiers with materials generated from USDA-ARS GEM Project

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Amylomaize VII, a class of High Amylose Maize with at least 70% of the kernel starch composed of the linear amylose polymer, has had numerous food and industrial applications including the manufacturing of biodegradable plastics, adhesives and candies. More recently it has been found to be a significant source of resistant starch, a pre-biotic, that increases populations of beneficial micro-flora in the lower digestive system in humans, thus, improving health in many ways, among them being of most interest, the suppression colorectal cancer cell formation. We found that a novel recessive starch branching enzyme 1a (sbe1a) likely plays a significant role in the presence of the ae allele in elevating starch amylose from ~55% to >70% in the germplasm release 'GEMS-0067'. A series of gene specific PCR-based marker were designed in order to identify the putative sbe1a::gm67 allele in GEMS-0067, for the purpose of initiating a marker-based selection protocol to allow a more rapid conversion of GEM releases previously selected for yield and agronomics to Amylomaize VII parent lines. Our studies demonstrate how the USDA GEM project can simplify efforts to incorporate biodiversity in commercial maize breeding program, especially when selecting for value added traits

Funding acknowledgement: United States Department of Agriculture (USDA), Germplasm Enhancement of Maize (GEM) program, North Central Plant Introduction Station

P205

Breeding for Healthier Short-Season Maize

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Most of the maize grown worldwide is used for food and feed. However, one primary limitation of maize is the insufficient amounts of essential amino acids to satisfy the nutritional requirements of monogastric animals, including humans. Lysine, methionine, and cysteine are the most limiting amino acids in feed ingredients. The long-term improvement of quality protein traits in maize will provide the next generation of healthier maize products with higher levels of these amino acids, and eventually will add value to the crop. Forty-seven quality protein maize (QPM) lines were crossed to elite early maturing inbred lines to identify high protein quality short-seasoned maize genotypes. Quality traits were screened using a near infrared grain analyzer. Genotypes with high protein quality were identified, and will be crossed to an industry tester for early generation hybrid combinations. Incorporation of protein quality traits to adapted inbred lines will provide competitive sources for healthier maize products in short-season environments.

P206

Changes in Plant Morphology in Recurrent Selection Programs in the Iowa Stiff Stalk Synthetic Population

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The maize plant phenotype has changed a great deal through the era of hybrid maize production. Some of the observed changes such as upright leaf angle, silking-anthesis interval, and tassel branch number, have well understood contributions to improved grain yield in modern hybrids. However, less is known formally about indirect selection responses for these phenotypes in the context of recurrent selection programs. The objective of this study was to determine if recurrent selection for agronomic performance in Iowa Stiff Stalk Synthetic (BSSS) population has changed important plant phenotypes. Thirty synthetic populations representing a total of 29 cycles of recurrent selection in three recurrent selection programs in BSSS were evaluated in four Iowa locations in 2008 and 2009. The most consistent changes observed across selection programs were for phenotypes that increase light penetration into the canopy, including flag leaf angle, flag leaf size, and tassel branch number. Light-interception phenotypes had more consistent responses in the populations per se evaluated here than agronomic traits selected on a testcross basis in the recurrent selection programs. Selection responses for morphological phenotypes in populations per se suggested these phenotypes may have much simpler inheritance than typically assumed for grain yield.

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P207

Characterization of Domestication QTL on Chromosome Five of *Z. mays*

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QTL controlling the phenotypic differences between maize and its progenitor, teosinte, are concentrated in five or six regions of the maize genome. One of these regions on the short arm of chromosome five (T5S) was chosen for further study. A nearly isogenic line (NIL) containing this region of teosinte chromosome five differs from the recurrent parent, maize inbred W22, for many plant architecture and ear traits. The broad effects of this teosinte chromosome segment could be interpreted as a single highly pleiotropic gene, or multiple linked genes spread across the segment, each with effects on just one or a subset of the traits. In an effort to study this question, the teosinte chromosome five NIL was backcrossed to W22, selfed for six generations, and a collection of 259 nearly isogenic recombinant inbred lines (NIRILs) was recovered. The NIRILs were genotyped at microsatellite and indel markers across the introgressed segment and phenotype data for domestication and other traits was collected for three replicates over two years. We found evidence for approximately 30 QTL spread across the introgressed region affecting all measured traits. The results suggest at least five independent regions of the introgression control the various domestication traits surveyed. Of these five regions, all have QTL for at least four different traits. Two of these regions are particularly pleiotropic with evidence for five, high LOD QTL. These results argue against the presence of a single pleiotropic domestication gene on chromosome five, and show that there is a minimum of five pleiotropic genes within the introgressed chromosome segment. Positional cloning efforts are underway to identify the genes responsible for two of the larger QTL.

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P208

Comparison of Inbreeding Depression between Diploid and Tetraploid Maize

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Inbreeding depression is the decline in vigor of inbred individuals compared with their non-inbred counterparts over several generations of selfing. Previous studies on inbreeding depression in alfalfa and maize tetraploids suggested that tetraploid and its comparable diploid has similar inbreeding depression rate. On the simple hypothesis that homozygosis of recessive detrimental alleles conditions inbreeding depression, this result if not predicted. To further test this hypothesis, inbreeding depression rates were compared among a set of maize diploid and tetraploid hybrids produced from crosses among diploid and tetraploid inbred lines of four genetic backgrounds, A188 (2n, 4n), Oh43 (2n, 4n), B73 (2n, 4n), and W22 (2n, 4n). These F1 hybrids include A188/Oh43 (2n, 4n), W22/B73 (2n, 4n), A188/Oh43 (2n) x B73/W22 (2n), B73/W22 (2n) x A188/Oh43 (2n), Oh43/A188/W22/B73 (4n) as well as 10 other diploid hybrids. Each F1 hybrid line was selfed for seven generations. Progenies from four selfing generations (S1, S3, S5, and S7) were used to collect data of measures for nine plant characters including height at four and six weeks, adult height, leaf length and width, ear length, tassel branch number, days to anther and silk emergence. Field experiments were conducted at Columbia, MO in the summers of 2008 and 2009 using a randomized complete block design. ANOVA and linear regression analyses revealed that inbreeding depression occurred in all measured characters and is affected by genetic background, ploidy and their interaction. The rates of inbreeding depression in tetraploid lines are faster than predicted based on the change of allele homozygosity in a population over the course of selfing. Indeed, the trajectory of inbreeding depression is very similar between tetraploid and diploid of the same genetic background. These results suggest that allelic dosage change may play a more important role than homozygosity to account for the inbreeding depression in tetraploids.

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P209

Comprehensive Analysis of the Genetic Architecture of Maize Kernel Composition in NAM using Joint-Linkage Mapping and Genome Wide Association

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Quantitative trait loci (QTL) for maize kernel composition traits starch, protein, and oil have been reported in several studies. The majority of these studies were restricted to analyzing biparental populations, thereby limiting mapping resolution and genetic diversity. The maize nested association mapping (NAM) population is a reference design with 26 founders and 5000 RILs. The NAM design simultaneously exploits the strengths of both linkage analysis and association mapping, and integrates natural diversity and genomics technologies. Seed from seven grow-outs of NAM was analyzed by near infra-red (NIR) spectroscopy to estimate starch, protein, and oil content. Heritability for the three traits ranged from 0.83-0.86, indicating that the majority of phenotypic variation is explained by genetic effects. Joint stepwise regression was used to fit a family main effect and markers nested within families. Results indicate NAM kernel composition is controlled by 21-26 QTL for each trait, explaining 59-69% of the phenotypic variation. No epistasis was observed at the NAM-population level. Additionally, we conducted a genome-wide association study (GWAS) using 1.6 million maize HapMap SNPs in order to detect SNPs associated with kernel composition. Several significant SNPs were immediately adjacent to DGAT1-2, a gene previously demonstrated to regulate oil composition and quantity. Results in NAM were verified in an association panel comprised of 282 inbred lines, both by using a genome scan using the 55K Maize Infinium Array and testing specific candidate genes. Identification and evaluation of kernel composition genes will enable further improvement of maize for food, feed, fuel, and industrial applications.

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P210

Detection of quantitative trait loci (QTL) controlling the bouquet-ear trait in maize

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Apical dominance is an important agronomic trait selected during maize domestication. In cultivated maize varieties, a single ear at the uppermost ear bearing node is the major sink for resources partitioned to grain yield. While formation of additional ears on lower nodes on the main stalk is common; certain stocks are prone to forming clusters of ears at a single shank or node, called bouquets. Bouquet ears are usually barren resulting in a substantial potential yield loss. The cause of the apparent loss of apical dominance in the ear branch remains unclear. In the Florida environment, the bouquet ears occurred frequently in the sweet corn inbred, P39. Thus, the B73xP39 nested-association-mapping (NAM) population was used to detect quantitative trait loci (QTL) controlling this trait. A set of 165 RI's were scored for numbers of tillers, lateral branches and bouquet ears as separate traits. QTL mapping was performed using six distinct combinations of the three branching phenotypes. Nineteen QTL controlling apical dominance traits were detected. As expected, a major QTL for tiller and branch number coincided with the location of *TB1* locus on the long arm of chromosome 1 validating our mapping results. Four QTL's that were specific for the bouquet trait were identified on chromosomes 1, 3, 7 and 10. Additionally, eleven out of the nineteen QTL's were specific for lateral branch growth; whereas, four QTL's controlled tiller development. Hence, a key finding is that the genetic basis for bouquet ear formation is distinct from pathways controlling tiller and lateral branch number. Candidate genes identified for bouquet QTL's provide insight in to the roles and integration of auxin, cytokinin and strigolactone signaling mechanisms in control of three distinct branching processes. Tests of these hypotheses are underway.

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P211

Development of Functional Molecular Markers of SbeI and SbeIIb for High Amylose Maize Germplasm Line GEMS-0067

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The development of high amylose corn in public sector has been hindered by the lack of high amylose germplasm available to breeders and difficulties to measure amylose content. GEMS-0067 is the first public germplasm that possesses recessive amylose-extender (ae) gene and modifer gene(s) and raises amylose level to over 70%. Several lines of eveidence suggested that SbeI is one of the major modifier genes for high amylose content. We developed and validated functional co-dominant molecular markers of SbeI and SbeIIb for GEMS-0067 that were able to distinguish AeAe, Aeae, and aeae genotypes. These simple, sequence-specific, PCR-based, low cost markers amenable to larger numbers of plant samples, make them potentially viable for marker-assisted selection in practical high amylose corn breeding programs. GEMS-0067 was of mixed heterotic derivation. Our result clearly showed that GEMS-0067 was genetically more similar to Mo17 and was clustered into NSS heterotic group based on 25 SSR profiles. The information provided here would facilitate the development of high amylose corn.

P212

Development of high through put analytical method for association maize and NAM of vitamins and essential amino acids in Maize seeds

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Maize is the most widely grown crop in the Americas with 332 million metric tons produced annually in the United States alone. Maize has served for many years as the key model for plant genetics in crop systems. Maize has enormous genetic diversity with the average nucleotide diversity of coding regions between any two maize lines being 2- to 5-fold higher than other domesticated grasses. Moreover, intragenic linkage disequilibrium (LD) rates rapidly decline to nominal levels within 2 kb in diverse maize inbred lines. This genetic diversity is complemented by tremendous phenotypic diversity which makes maize a powerful system for studying the genetic architecture of complex traits. Recent developments in maize genetic and genomic resources, particularly the NAM population, allows for high-resolution, genome-wide genetic study of traits, while association mapping panels enable the contributions of known and novel candidate genes to natural variation to be assessed. Identifying favorable alleles for improved micronutrient content in maize grain will enable downstream application in maize-based components of the diet, ultimately benefiting human and animal nutrition and health in both developed and developing countries. The first step to achieve such a goal is to develop accurate, rapid, large-scale high throughput analytical methods for detecting a wide range of target compounds simultaneously in association panels and NAM. We developed and validated a 13 minute HPLC method using an internal standard ratio method that identifies and quantifies the 12 most abundant carotenoids and all tocochromanols in maize seed (linear from 10 to 500 ng and with r2 >0.98). A 5.6 minute, internal standard ratio LC_MS/MS method has also been validated for the identification and quantification of 18 protein amino acids using multiple reaction monitoring (MRM) transitions selective to each amino acid. A 6 minute, internal standard ratio LCMS methodology has been validated for extracting and quantifying four B vitamin complexes (nicotinamide, nicotinic acid, pyridoxal, pyridoxine, pyridoxamine, riboflavin and thiamin). These advanced analytical methods are generating a vast phenotypic dataset for these essential micronurtients in maize seed that will be central for quantitative genetic analysis in this project and future application to nutritional deficiencies.

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P213

Difference of Pb and Cd accumulation in 19 elite maize Inbred lines and application prospects

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The accumulation of heavy metal in crop grains has been focused on many researches. However, the stems and leaves of maize are often used as silage fodder for livestock, which can increase the risk of heavy metal movement into the food chain. In this study, Pb and Cd accumulation in the stems and leaves of 19 representative elite maize inbred lines and three hybrid varieties was investigated at the seedling stage. The results demonstrated significant differences among inbred lines for accumulation of heavy metals, implying that the Cd concentrations are significantly correlated between the male parents and their hybrids. Some inbred lines with low Pb or Cd accumulation have been selected for crossbreeding with low Pb or Cd varieties. Some inbred lines accumulating high levels of Pb and Cd in this experiment could be suitable phytoremediation species for soil bio-remediation.

P214

Dissection of High Temperature Tolerance Traits of Maize

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Heat waves (high temperature stress) often occur sporadically during maize growing season on the US High Plains, causing irreversible damage to maize developing leaves (leaf firing) and destructive desiccation of tassel tissues (tassel blasting) and resulting in reduction in photosynthesis, pollen shed, kernel numbers, and total grain yield. Understanding high temperature adaptation mechanisms in maize plants is crucial to the success of developing high temperature tolerant hybrids/varieties. However, little is known about the mechanisms of high temperature tolerance in maize. We have initiated a collaborative project aims at 1) dissecting the regulation and genetic control of high temperature tolerance traits in maize and 2) identifying molecular markers for marker-assisted selection of high temperature tolerance traits in maize breeding programs. Field evaluation shows great genetic variation in high temperature tolerance among maize germplasm. Maize inbred lines with contrasting high temperature tolerance phenotypes at different developmental stages were selected to generate mapping populations. We have also identified 2 publicly available RIL populations suitable for high temperature tolerance study. Genetic analysis suggests at least four independent genetic traits contributing to the variation in high temperature tolerance in field-grown maize. Preliminary study shows five QTLs for one of the high temperature tolerance traits.

Funding acknowledgement: United States Department of Agriculture (USDA)

P215

Effect of allele frequency changes on the ability to detect loci of genetic importance in the Golden Glow maize population long term selection program

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Long-term selection programs are a resource for QTL discovery. The ability to detect QTL in long term selection programs will depend on factors such as initial allele frequency, effect size, gene action, linkage disequilibrium, effective population size, and selection intensity. The objective of this study is to determine the effect of allele frequency changes produced by selection on the ability to detect quantitative trait loci (QTL) of varying effect sizes. To investigate the effectiveness of this technique, the Golden Glow maize population [GG(MP)] was utilized as a model. The GG(MP) has been selected for prolificacy, or number of ears per plant, for thirty generations. Between 4,250 and 14,250 individuals were evaluated and approximately 200 of those were selected in each cycle of selection. A study on cycle 23 of this population identified four QTL associated with total number of ears per plant. Two of these four QTL displayed partial-dominance and two displayed overdominance. A selection model allowing for drift was developed. When modeling the ability to detect the two partially-dominant QTL undergoing directional selection we found that when the favorable allele was at an initial frequency of at least 0.01 and not more than 0.8, the probability of correctly detecting either of the two was greater than 99%. Moreover, the two QTL that displayed overdominance, a phenomenon which leads to balancing selection, were also predicted to be largely detectable when initial frequencies were not at intermediate values. Lastly, modeling potentially detectable QTL from the allele-frequency approach in Golden Glow suggests that there is the ability to find loci as small as 1/10 the size of the additive effects of the known QTL when initial allele frequency is between 0.01 and 0.8. We conclude that when certain allele frequency conditions are met in the initial population, the power to detect QTL of varying sizes is high for a population that has undergone several generations of selection.

Funding acknowledgement: Monsanto Fellowship

P216

Effect of model and training population on genomic selection for multiple traits in maize

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Genomic selection attempts to maximize marker-based prediction accuracy for complex traits by modeling all marker effects simultaneously. Various types of models exist for modeling marker effects. These models make implicit assumptions about the underlying genetic architecture. Clearly, different traits vary in their genetic architecture. Regarding multiple traits, a natural question would be how to combine multiple trait predictions into an index. Training population composition and size also affects prediction accuracy. Is this effect consistent across different types of traits? Simulated and real datasets from a biparental maize population were analyzed to help answer these questions.

P217

Effective Recombination in Plant Breeding and Linkage Mapping Populations: Testing Models and Mating Designs

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Effective recombination events are recombination events that result in novel genetic combinations which can be directly observed; always less than the actual and expected number of recombination events. Altering population designs can improve effective recombination which is often a limiting factor in breeding and genetic linkage mapping. Using a simulation approach, this study sought to model and quantify effective recombination under various population designs. The number of markers needed to observe all effective recombination events and the distribution of the expected number of effective recombination events were then estimated. Three recombination models were used including one with recombination rates fit to the large *Zea mays* L. nested association mapping (NAM) dataset. Strong evidence was found in the empirical NAM dataset supporting a two- pathway modified Poisson model of recombination events with separate rate λ for each chromosome, reflecting the significant differences in effective recombination rates found across chromosomes. A positive linear relationship between the mean number of effective recombination events per generation and genomewide heterozygosity was observed. Primarily because of this phenomenon, dihybrid and doubled haploid populations increased the number of effective recombination events per generation when compared to traditional biparental recombinant inbred line populations. This study will be useful for quantitative geneticists and breeders in identifying efficient production of effective recombination events as well as researchers simulating recombination.

Funding acknowledgement: United States Department of Agriculture (USDA)

P218

Fine Mapping and Field Evaluation of a Quantitative Trait Locus conferring Resistance to Southern Corn Leaf Blight

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Southern leaf blight (SLB) caused by the fungal pathogen *Cochliobolus heterostrophus* (anamorph = *Bipolaris maydis*), is a common disease of maize in southeastern US, as well as many hot and humid tropical and subtropical areas in the world. Most of the disease resistance used in maize is quantitative in nature; however, quantitative disease resistance remains poorly understood. We have identified a strong QTL for SLB resistance at the tip of the short arm of chromosome 6 in maize. Specific objectives of this research include fine-mapping the region where this QTL is located (namely 6A), cloning the gene that accounts for this effect, and evaluating its yield and fitness effects under both high and low disease pressure for possible future use of this resistance. Preliminary growth chamber phenotyping experiments showed that 6A segregates as a single recessive resistance gene, and can be scored in growth chamber experiments on a single plant basis. 168 F_2 individuals and over 500 $_{F2:3}$ families were phenotyped, and genotyped with SNPs markers. We narrowed down the region of interest to approximately 0.3 Mb, encompassing 15 genes. Currently, candidate genes are being analyzed. To evaluate the effect of the genes on fitness and yield, we have developed isohybrid pairs by crossing B73 with/without locus 6A to several inbred lines (testers). We have found significant differences in disease resistance between the isohybrid pairs in some pedigrees. Yield trials are in process to study the influence of the presence or absence of 6A locus on agronomic traits and disease.

Funding acknowledgement: United States Department of Agriculture (USDA)

P219

Functional Stay-green: Abiotic Stress Tolerance in Maize

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The ability for annual crop species to delay senescence or "stay green" through the grain fill period has been associated with increased productivity. Functional stay-green describes plants that can also continue photosynthesis late into the growing season. Studies in grain sorghum have shown a correlation between the stay-green phenotype and drought tolerance during the grain fill stage. Diverse germplasm also exists in maize for this delayed senescence characteristic. Our goal is to investigate functional stay-green through multiple approaches. Mo20W is a maize inbred line evaluated for functional stay-green in abiotically stressful environments. Mapping populations were created by crossing Mo20W with B73 and Mo17 to produce two separate sets of BC2F4 recombinant inbred line populations (RILs) with B73 and Mo17 as the recurrent parents. Relative greenness and FvFm fluorescence were used to determine visual stay-green and functional stay-green, respectively. Mo20W exhibits a functional stay-green phenotype when compared to B73 and Mo17 during late grain fill. Results from the Garden City, KS location show phenotypic variation within each mapping population for visual and functional stay-green at four weeks post-flowering. Preliminary data from a bulk segregant analysis indicates multiple loci responsible for the functional stay-green phenotype. We are also evaluating functional stay-green in available natural diversity by utilizing the Nested Association Mapping (NAM) population in maize. Hybrids were created from NAM inbred lines, selected for uniform flowering time using a common tester. These hybrids were evaluated for stay-green across four diverse environments. Joint-QTL analysis indicates the presence of two major QTL for stay-green.

Funding acknowledgement: AFRI

P220

Functional mapping of maize phosphate responses

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Genotype x Environment effects are not just an inconvenience to be controlled, but may describe the very traits we are interested in. Dynamic properties such as *efficiency* , *stability* , *tolerance* , *responsiveness* , or *reliability* can only be defined by reference to the relevant dose-response relationship, or *reaction norm* , a description of the phenotype-environment relationship. And yet, how do we compare dynamic properties among varieties? What is their genetic basis? Can they be optimized by selection or manipulation? *Functional mapping* (1, 2) addresses these questions by estimation of model parameters that describe a given reaction norm; it is these estimates that then form the basis for comparative assessment of genotypes, or for genetic mapping. Significantly, this approach recognizes the inadequacy of characterization under a single environmental condition, or of analysis based on indices derived from arbitrary "high" and "low" treatment levels. We are using a functional mapping approach, in combination with study of candidate genes and reverse genetics, to investigate maize phosphate responses, an area of ever increasing importance in the face of diminishing global phosphate reserves. Using a solid-phase, slow-release phosphate system, we are quantifying and modelling a number of traits in young plants across a range of phosphate availabilities – ranging from biomass and morphology to whole transcriptome gene expression. We will present preliminary data from analysis of reference lines, mapping populations, and diversity panels.

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P221

GEM – Meeting the Challenge of Maize Diversification by Capturing Useful Alleles and Traits from Exotic Germplasm

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The Germplasm Enhancement of Maize (GEM) Project was conceived as a public and private research partnership dedicated to diversifying the genetic base of U.S. maize production. It is coordinated by USDA-ARS and university personnel located at Iowa State University and North Carolina State University. Working with unadapted germplasm presents serious challenges, including photoperiod sensitivity, standability and other agronomic issues, and severe defects resulting from inbreeding depression. These issues make it difficult for traditional, conventional breeding programs with short cycle times to adapt exotic germplasm and derive lines that can be used effectively in commercial breeding programs. GEM industry cooperators provide private sector germplasm used to introgress exotic germplasm via a standard protocol. Public and private sector researchers share responsibility for breeding and testing activities. Short daylength is necessary to accomplish many initial breeding crosses, and photoperiod control structures or tropical winter nursery locations are used to facilitate these efforts. Phenological, agronomic, disease and insect resistance, and grain composition or quality trait research results are processed by the coordinating personnel and publicly shared. In collaboration with the Iowa State Doubled Haploid Facility (http://www.plantbreeding.iastate.edu/DHF/DHF.htm), dihaploid lines are generated in addition to conventionally generated GEM Project lines to provide a set of adapted lines representative of the races of maize. These lines provide a rich resource for an array of modern research and development objectives. Project information can be found at http://www.public.iastate.edu/~usda-gem/ .

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P222

Genetic Regulation of Grain Protein Concentration in Maize Following Long Term Selection

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The Illinois Long-term Selection Experiment for grain protein and oil concentration has produced populations, the Illinois Protein and Oil Strains, with the known phenotypic extremes for theses traits, and also illustrates the nature of responses to phenotypic selection. Here we provide an update to the experiment, including the introduction of new chemical analytical procedures, assessment of methods for estimating genetic gain, and the recent initiation of reverse selection experiments from cycle 103 of Illinois High Protein (IHP). Prior genetic mapping studies suggest that the response to selection for grain protein concentration in this experiment is dependent upon the cumulative action of many genes with small phenotypic effects. An alternative theory explored here is that the response depends on quantitative expression variation of a few major regulators. Analysis of crosses between inbreds derived from IHP and Illinois Reverse High Protein (IRHP) provides evidence that the response to selection in IRHP is due to one or a few loci with main effects, which is associated in part with biased frequencies for variant alleles of the *ASPARAGINE SYNTHETASE3* gene. Underlying the dramatic divergence of protein concentrations in the Illinois Protein Strains (IPS) are changes in α-zein gene expression. To identify additional genes that may regulate α-zein gene expression and protein accumulation in the IPS, genetic mapping studies are employed utilizing expression levels of a zein-mRFP reporter transgene as an easily measured, nondestructive phenotype. When *Floury2-mRFP* is crossed to inbred lines derived from crosses among the IPS, the resulting ears exhibit varying degrees of *Floury2-mRFP* expression that can be quantified by the pink coloration of the kernels and correlate with protein concentration, making the *Floury2-mRFP* transgene a useful tool for identifying regulators of α-zein gene expression.

P223

Genetic Analysis of Maize Kernel Color Using Segregation, Bulked Segregant, and Nested Association Mapping Analyses

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Carotenoids are very important nutritionally, as sources of pro-vitamin A for the developing world, and as antioxidants important for eye health for both developing and developed country populations. Simple visual selection for darker orange color has been associated with an increase in total carotenoids. Visual scoring is much less expensive and time-consuming compared to other quantitative carotenoid measurements such as HPLC and NIR. Segregation analysis using visual scoring of S1 kernels from a four-parent synthetic revealed that colors segregate in a relatively simple mendelian manner and that very few genes are involved in the conversion from yellow to orange kernel color. Bulked Segregant Analysis (BSA) was performed on bulks of light orange and dark orange kernels from an F2:3 mapping population. The kernels making up the bulks were scored visually and the genotyping was done using an Illumina MaizeSNP50 chip. Paired t-tests were performed to detect genetic differences between the bulks. Several loci were considered statistically different between bulks and some mapped to regions of the genome near known carotenoid biosynthesis genes. QTL analysis was performed on 10 Nested Association Mapping (NAM) families segregating for yellow and orange kernel color. The results of both individual family and joint family analysis further indicated that only a few genes are largely involved in the conversion from yellow to orange kernel color. Markers near two logical candidate genes, phytoene synthase 1 (psy1) and lycopene ε-cyclase (lcyε), were significantly associated with the trait in several families as well as in the joint analyses. Other QTL having smaller effects on kernel color are located near known carotenoid biosynthesis genes including whitecap 1(wc1), β-carotene hydroxylase 1(crtRB1), ζ-carotene desaturase 1 (zds1),and yellow 8 (y8).

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P224

Genetic basis of plant architectural development in grasses – insights from foxtail millet

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The breeding of efficient plant architectures for biofuel production requires the understanding of the genetic basis of plant architecture. In grasses, plant architecture can be decomposed into branching structure modules and accompanying morphological and anatomical differentiation. Maize has been particularly productive in elucidating the genetic control of vegetative branching in grasses, and comparative genomics has allowed information from maize and other model systems to be applied to other grass systems, such as the foxtail millet – green millet (*Setaria italica – S. viridis*) system. We are particularly interested in this system because of its pronounced variation in branching patterns, and because of completed genome sequences for both species. Multiple trials of mapping populations from a cross between green and foxtail millet have indicated major QTL for branching and height, some co-occurring with candidate genes such as the *teosinte branched1* and *barren stalk1* orthologs. A recent set of greenhouse and field trials with an F7 RIL population recapitulates those results, but finds that QTL can vary between different growth stages, suggesting that some genomic regions have a constant influence in one or more traits during the life span of the plant while others seem to be time/developmental stage specific. Our results provide insights into the tradeoffs taking place throughout the development of plant architecture during the plant's life span, suggesting that mechanisms of genetic control of branching phenotypes vary over the life cycle of the plant.

Funding acknowledgement: Department of Energy (DOE), Oklahoma Center for the Advancement of Science (OCAST)

P225

Genetic mapping approaches to identifying N utilization genes in maize

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Nitrogen utilization is of special significance to maximizing yields, reducing nitrogen fertilization use, and improving the energy balance for agricultural production. Studies in model plant species such as Arabidopsis have implicated a number of potential molecular networks that contribute to nitrogen utilization, but these have yet to be linked to genetic variation for N utilization in crops such as maize. We have used a genetic mapping approach to characterize the genetic architecture of nitrogen utilization in maize and to identify genes that control this agriculturally important trait. QTL mapping in hybrids derived from high resolution mapping populations, grown under N-limiting and N-sufficient conditions in the field, identified a set of robust QTLs across the genome controlling important agronomic traits and physiological indicators of N utilization. Several QTL hotspots were identified, including some where QTL for agronomic N utilization, amino acid metabolites, and expression QTL (eQTL) for genes controlling N metabolism are localized between the same flanking markers. Fine mapping studies further narrowed down these promising QTLs to one or a few candidate genes. Our results confirm the importance of asparagine cycling as a key pathway for N utilization in maize, identify additional genes affecting N utilization, and suggest allelic variants that could be directly exploited for improving N utilization in maize.

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P226

Genome-wide Association Mapping in the Maize NAM Population Permits Precise Identification of Loci Conditioning Southern Leaf Blight Resistance

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The maize Nested Association Mapping (NAM) population, a collection of 5000 recombinant inbred lines (RIL) derived from crosses between B73 and 25 founder inbreds, is a high resolution platform for mapping quantitative traits in maize. We identified 32 quantitative trait loci (QTL) in the NAM population that govern quantitative resistance to Southern leaf blight, caused by Cochliobolus heterostrophus. Using the first generation maize haplotype map, we projected founder parent genotypes at 1.6 million SNPs onto the full set of NAM lines based on their genotypes at 1,106 SNP markers. Using two different association analysis methods, we identified 245 SNPs that significantly contribute to SLB response. Further model selection, with the goal of replacing the 32 QTL with biallelic SNP effects, allowed for reduction of the model to 51 SNPs and three QTL. Genes in which these SNPs reside, and those adjacent, are candidates for SLB resistance genes. We were able to classify many of these genes based on their similarity to genes encoding proteins involved in plant disease resistance, e.g. R genes, NPR1 (nonexpressor of pathogenesis-related genes 1), and genes that interact with NPR1. Because moderate to low linkage disequilibrium exists in the NAM population, the addition of approximately 24 million more SNPs from the second generation haplotype map will increase resolution, possibly to the gene level.
We will also present data on the fine mapping and characterization of other maize QTL for resistance to southern leaf blight, grey leaf spot and northern leaf blight. We will present our latest results on multiple disease resistance (MDR) loci in maize. Our analyses have suggested that multiple resistance to the three diseases studied here is often due to the accumulation of disease-specific genes but we also have evidence for the existence of genes with pleiotropic effects that condition MDR.

P227

Genome-wide association study of maize leaf using the second generation of maize HapMap

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Using 1.6 million SNPs from the first generation of the maize HapMap, we previously conducted a genome-wide association study (GWAS) on the maize nested association mapping (NAM) population for three leaf architecture traits (leaf angle, leaf length and width) that are important for the adaptation of maize to high density planting. We identified hundreds of associations for these three leaf traits and, in particular, found that variations at the liguleless genes have contributed to more upright leaves. However, the fact that HapMap v1 SNPs are in high LD with each other only 34% of the time led to decreased power and resolution to detect and dissect QTL. Additionally, the contribution of structural variation in the form of copy number variation (CNV) was not evaluated. With the recent availability of the second generation of maize HapMap that includes 50 million SNPs and CNVs, we are now able to further dissect the QTLs for these leaf traits, decompose the detected associations and estimate the relative contributions of CNV versus SNP in determining phenotypes. Using this new HapMap v2 dataset in conjunction with the HapMap v1 SNPs, more QTLs are dissected and the allelic architecture of QTLs is determined more precisely. We further found that CNV, especially genic CNV, explained a substantial proportion of phenotypic variation, suggesting their functional importance in determining the tremendous phenotypic diversity of maize. This advance represents an important step toward further dissecting the genetic architecture of maize complex traits and cloning QTL underlying the genetic architecture.

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

P228

Genomewide Selection to Introgress Exotic Traits into Adapted North American Germplasm

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Exotic germplasm has been underutilized in maize (*Zea mays* L.) because of the extensive pre-breeding required. Theoretical and practical studies have shown that 5-10 cycles of phenotypic testcross selection, which is equivalent to 10-20 years of breeding work, are generally required to improve exotic maize populations. In contrast, simulation studies have shown that genomewide selection could shorten pre-breeding from 10-20 years to less than 4. This reduction in time is achieved by the use of molecular markers to select for complex traits in a year-round nursery or greenhouse, where phenotypic measurements are not meaningful but where marker data remain unchanged. Furthermore, because genomewide selection acts on the entire genome, it can select for simultaneous integration of multiple traits. The objective of this study is to compare the efficacy of genomewide selection and phenotypic backcrossing to introgress dwarfing while maintaining agronomic quality. The exotic donor is a line derived from COPOP1, an open-pollinated Canadian population that is a source of dwarfing, density tolerance and earliness. Two non-dwarf lines, PHG50 (developed by Pioneer Hi-Bred) and LH74 (developed by Monsanto), serve as the adapted parents. Evaluation of F3-testcrosses shows significant variation for both plant height (113-232 cm) and agronomic traits (yield 2.9-9.2 tons/hectare). We confirmed that COPOP1 carries the major dwarfing gene *rd1*. From each cross, 10 F3 families that best combined short height and good agronomic performance were selected for advancement. An additional 4 cycles of selection will be performed using performance predictions based on marker scores alone. Concurrently, phenotypic backcrossing is being done on the same material with the shortest plant at flowering backcrossed to the adapted parent. Once selection is complete, testcrosses of material from each cycle will be evaluated to compare the genetic gain from phenotypic backcrossing and genomewide selection for both the exotic trait (dwarfing) and agronomic traits.

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P229

High resolution mapping of B73-Mo17 near isogenic lines

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Recombinant inbred lines developed from the maize inbreds B73 and Mo17 have been widely used to discover quantitative trait loci (QTL) controlling a wide variety of phenotypic traits. These two parents were used to produce a set of near-isogenic lines (NILs) with small regions of introgression into both recurrent backgrounds. A novel array-based genotyping platform was used to score genotypes of over 7,000 loci in 100 NILs with B73 as the recurrent parent and 50 NILs with Mo17 as the recurrent parent. This population contains introgressions that cover the majority of the maize genome. The utility of this population for the validation and fine-mapping of phenotypic QTL was demonstrated by the identification of several previously described QTL for plant height. The NILs displayed an excess of residual heterozygosity relative to the amount expected based on their pedigrees and this excess residual heterozygosity is enriched in the low-recombination regions near the centromeres. The genotyping platform provided the ability to survey copy number variants that exist in more copies in Mo17 than in B73. These Mo17-specific duplications were studied in the NILs in order to determine whether the additional Mo17 copies were located near the original locus (linked) or elsewhere in the genome (unlinked). The majority of Mo17-specific duplications are located in unlinked positions throughout the genome.

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P230

High-throughput and Quantitative Genotyping Applications in Economically Important Crops

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Recent technology developments in Next Generation Sequencing allow researchers to generate a huge amount of data and to discover many new genetic variations in crop species. . The Single Nucleic Polymorphism (SNPs) discovered are being used for a variety of important applications including marker assisted breeding, crop strain validation, candidate genetic marker evolution, and Quantitative Trait Loci (QTL) mapping. These applications require a high-throughput targeted genotyping platform which provides high quality data at very low cost. In this poster, we demonstrate the use of Sequenom's MassARRAY® technology in maize, wheat, rice, and sugarcane genotyping. The quantitative nature of the iPLEX® Gold genotyping assays enables users to conduct polyploid genotyping as well as generating statistical analyses such as Bulk Segregant Analysis (BSA). The flexible MassARRAY® system is widely applicable to many crop species and applications.

P231

Hybrid Vigor in the Maize Nested Association Mapping Population

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Hybrid vigor or heterosis, is defined as the increased fitness of a hybrid over its parents. Since their humble origins, hybrids have not only made enormous contributions to the field of genetics; they have also had a tremendously important role in the improvement of maize yields over the past hundred years in the U.S. and other developed nations. In the 1930s when corn hybrids were first commercially grown in the U.S., corn production was 53 million Mg. This number has increased over the years to 332.7 million Mg due to improved hybrid development and increased plant density. The interactions between genes possibly responsible for heterosis are believed to be explained by dominance, overdominance, and epistasis, or a combination of all or part of the above. Today, hybrids are purposefully developed in many species, both plants and animals, and yet the mechanism underlying heterosis still remains a mystery.

Here we utilize hybrids created by crossing a subset of the maize Nested Association Population (NAM) population and PHZ51, a nonstiff stalk, to determine the role of genetic dominance in maize yield, plant height, and flowering time. Prior studies of the NAM population and analysis of the genetic diversity characterized in the first generation maize haplotype map suggest that there is increased residual heterozygosity in pericentromeric regions of the maize genome. From these observations we infer a direct relationship between recombination rate and residual heterozygosity. The primary goals of this study are to enhance our understanding of the physical and genetic architecture underpinning quantitative trait loci explaining variation in hybrid maize yields, and to provide an empirical foundation of data on which genomic selection models may be trained. After sufficient development and cross-validations, these modeling algorithms may be used to successfully predict hybrid maize yields worldwide. We will here present preliminary findings from this study.

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P232

Identification and mapping of branching modifiers in *ramosa* mutants

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ramosa1 encodes a C2H2 zinc finger protein involved in the suppression of lateral branch growth in maize inflorescences. Tassels of *ramosa1* mutants display increased branching and ears exhibit unorganized rows and/or lateral branching. The severity of the *ramosa1* mutant phenotype is largely dependent on the inbred into which the mutation is introgressed. For example, the phenotypes of *ramosa1* mutant alleles are stronger in B73 vs. Mo17 backgrounds. Using the IBM population, we exploited these phenotypic differences to map modifiers of ear branch number. Each RIL in the IBM-94 population was backcrossed to Mo17- and B73-introgressed *ra1-63.3359* to generate two sets of F1BC1 populations that segregated *ra1-63.3359*. For each population, ear-branch number was used to map modifiers of branching in the B73 and Mo17 backgrounds. Using this method, we were able to identify several loci in Mo17 and B73. Additionally, we employed an alternative strategy for mapping modifiers of branch number in *ramosa1* and *ramosa2* mutants using Bulk Segregant Analysis (BSA). In this experiment, F2 populations were generated using B73- and Mo17-introgressed *ramosa* mutant alleles. Bulked samples representing the weakest and strongest individuals of each F2 population were collected and marker segregation ratios compared using Sequenom MassARRAY. Several putative modifiers were identified using this method including one modifier that appeared in both *ramosa1*. and *ramosa2* populations. The results of these analyses will be presented along with preliminary results for the fine-mapping of modifier loci.

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P233

Identification of exotic alleles that confer native resistance to Western corn rootworm beetles

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Western corn rootworms (WCR) are currently the most damaging pests of maize, causing substantial global economic losses due to diminished yield and control costs. 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 evolved independently at least twice, and transgenic crop failures have been reported in each of the past several years. Significant research efforts to improve the efficacy and durability of insecticidal transgenes are ongoing, but there is also a need to identify and deploy native resistance (NR) mechanisms. A principle challenge is to identify and characterize the effects of NR alleles, so that the eventual goal of understanding the underlying NR mechanism(s) can be met. Through repeated evaluations of diverse germplasm accessions, two exotic landrace varieties with enhanced tolerance to WCR were identified. F_2 and backcross populations were generated separately for each exotic source for QTL mapping. These segregating populations were evaluated on several measures of resistance over two nursery seasons. Genotypes were collected for 170 SNPs distributed across the ten maize chromosomes. Several significant associations between genotype and phenotype were detected in both our F_2 and BC_1 populations. These findings demonstrate that allelic differences in the host confer varying levels of native resistance, and further, that these functional polymorphisms are experimentally tractable. We discuss the challenges of pursuing fine-scale mapping and detailed allele characterization in this plant-pest interaction system.

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P234

Identification of leaf trait related genes by genome-wide nested association mapping of maize leaf traits with HapMap and RNA-Seq SNPs

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To effectively dissect genetic components of complex traits, the maize nested association mapping (NAM) population with ~5000 recombination inbred lines (RILs) has been developed by crossing 25 diverse founders to a common parent, B73. This strategy combines the advantages of linkage analysis and association mapping. With genotyping data from 1106 B73-specific tag SNPs across RILs, about 100 QTLs related with three leaf traits (leaf length, leaf width, and upper leaf angle) were mapped by linkage analysis. To pinpoint causative genes underlying these QTLs, two dense SNP (dSNP) datasets were obtained for the NAM founders: 1.6 million maize HapMap SNPs from sequencing genomic DNA, and 0.9 million SNPs from sequencing mRNA extracted from shoot apical meristem of maize seedlings. Information of these dSNPs was projected from the founders to the RILs based on the genotypes of the flanking tag SNPs and the resulting SNP data were used in genome-wide NAM analysis with three leaf traits. Candidate genes underlying significant association signals were identified. To verify these results, several mutants containing insertion in these candidate genes have been selected from maize mutagenesis stocks for further genetic analysis.

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P235

Identification of natural variation in the root system architecture in the Nested Association Mapping (NAM) population

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Roots are the point of contact between plant and soil, and serve to acquire both nutrients and water. As such, the root system architecture (RSA) of a plant reflects the needs of the plant itself, the composition of the plant's environment, and ultimately, the plant's response to both. By identifying genes that control root development under either ideal or arduous conditions, it will be possible to create new varieties of corn with root systems better adapted to a particular situation. Here we present the great amount of variation observed in both 2D and 3D RSA traits in the 26 different varieties of corn grown under standard conditions. These are the same varieties used to created the nested association mapping (NAM) panel, and therefore part of this population will subsequently be used to perform QTL mapping experiments, first to locate and then to fine-map loci of potential scientific and agronomic interest. The plants themselves were grown in a gel system. This allowed for rapid and nondestructive imaging of the plants on multiple days. Computational methods were then used to phenotype both the 2D images themselves, as well as used to reconstruct 3D models of the roots, and in turn phenotype these as well. The data shown here demonstrate a wide spectrum in traits scored. Some varieties, such as B73, have shallow and compact root systems while others, such as Ki3, have have much deeper and spread out roots systems, demonstrating just some of the variability observed among the whole population.

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P236

Identifying genes associated with provitamin A and vitamin E content in maize kernel through association mapping

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Maize is a primary food source for millions of people in the developing world. However, this crop by itself does not provide adequate levels of vitamin A and E nutrition. Consequently, vitamin A deficiency (VAD) and to a lesser extent, vitamin E deficiency (VED) are serious problems that affect many people. Some examples of health-related issues that arise from these nutritional deficiencies include eventual blindness (from VAD) and neuromuscular problems (from VED). One solution for reducing VAD and VED is to increase the amount of provitamin A and vitamin E in the maize kernel. These compounds naturally occur in maize, specifically in carotenoids (e.g. β-carotene) and tocopherhols (e.g., α-tocopherol), respectively. Our research uses joint linkage-association mapping methodologies to identify genes and alleles associated with kernel carotenoid and tocopherol levels. Breeders can then use marker assisted selection to select on superior alleles and develop lines with increased vitamin content. Our work focuses on testing relevant associations for approximately 50,000 SNPs across the genome, as well as the strengths and weaknesses of testing imputed SNP genotypes at biosynthetic pathway genes. We use a mixed model to account for the confounding effects of population structure and the Benjamini and Hochberg procedure to adjust for the multiple testing problem. To date, we reconfirmed two biosynthetic genes that are associated with elevated provitamin A carotenoids, and one biosynthetic gene that is associated with α-tocopherol. Our ultimate goal is to identify enough genes to develop maize lines with 15 µg/g of β-carotene and 30 to 60 µg/g of α-tocopherol. These amounts should result in enough vitamin A and E to meet the Daily Recommended Intake (DRI) levels specified by the National Academy Panel.

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P237

Impact of weather and soil on the performance of hybrid maize in multi-environment trials

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Maize is the most widely cultivated crop in the United States. It is grown in fields representing diverse soils as well as continuously changing weather conditions. The diversity of these conditions can produce variation in performance across fields. Estimation of the portion of this variation that is due to interaction of genotype by environment (GEI) is important in identification of maize hybrids that are better adapted to specific conditions and/or broadly adapted across environments.

In this study, multi-environment field trials conducted using an augmented randomized block design was investigated to determine the effects of soil and weather on genotype performance and GEI. A number of statistical methods such as principal component analysis, and partial least square regression were used to estimate the GEI and possible causes of this interaction. In preliminary analyses, we observed that certain weather and soil factors during the pre-planting and vegetative phase of crop had significant influence on hybrid performance and variation in performance across locations. This information is being used to inform breeders, researchers, and farmers, about the magnitude and nature of GEI, causes of interaction, adaptation of a crop, and behavior of a crop in extreme environmental conditions.

Funding acknowledgement: Department of Agronomy Purdue University, Dow AgroSciences

P238

Improving grain yield and yield components via backcross procedure

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Four-consecutive backcross procedure with three local and three exotic maize lines was used for 1) determining the backcross generations at which a quantitative trait was recovered to a similar homozygous level of recurrent parent; 2) investigating how a quantitative trait has been transferred from exotic donors into local lines; 3) evaluating if the parental lines' mean, general (GCA) and specific (SCA) combining ability effects impact on the changes of the studied traits at different backcross generations. The results had shown that four backcross generations were necessary to make a new line recover to similar homozygous level of recurrent parent in all studied quantitative traits. The multiple quantitative traits from exotic donor line have been successfully transferred into the new developed line by the backcross procedure. Parental line's GCA effects had positive correlation with the means of the studied quantitative traits after four consecutive backcrosses, suggesting that lines with positive GCA effects from donor line were preferred to that with positive SCA effect for improving a quantitative trait because additive genes could be fixed in consecutive backcross generations.

P239

In-depth characterization of transgenic events carrying targeted insertions at the maize *ZmIPK1* locus

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In a previous study, we described targeted insertion of an herbicide tolerance transgene into the *ZmIPK1* locus in maize using zinc-finger nucleases (ZFNs)[1]. *ZmIPK1* encodes inositol-1,3,4,5,6-pentakisphosphate 2-kinase, an enzyme that catalyzes the final step in phytate biosynthesis in maize seeds[2]. Our continuing aim is to demonstrate the stability of ZFN-mediated genome modification and to generate data supporting regulatory advancement of events created using targeted gene insertions. Here, we describe analysis of such transgenic events. Three independent events, each carrying a single-copy insertion of PAT at the *ZmIPK1* locus, were advanced to the field nursery and subjected to multigenerational analysis of transgene segregation ratios, transgene DNA sequence, presence/absence of integrated ZFN elements, event border sequence composition, PAT protein expression, herbicide tolerance (glufosinate), and agronomic performance. From these analyses all three events were shown to meet expectations and to be isogenic at the *ZmIPK1* locus. Marker assisted introgression of the events into elite germplasm was also initiated toward the goal of carrying out yield trials to determine if *ZmIPK1* can be considered a preferred locus for additional future transgene insertion in maize without incurring agronomic penalty.

[1] Shukla et al. (2009) Nature 459:437-441

[2] Sun et al. (2007) Plant Physiol. 144:1278-1291

P240

Initial Characterization of *zagl 1*, a Target of Selection during Maize Domestication

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Although still modest in number, studies using genomic scans for selection are becoming more prevalent. Four such genomic scans in maize have identified 80 candidate loci involved in domestication and crop improvement. A highly significant candidate, *zagl 1*, is a MADs box gene that has previously been shown to associate with ear shattering, a key domestication trait. In this study, the function of *zagl 1* is investigated using transgenic overexpression lines, RNA interference lines, and recombinant chromosome near isogenic lines (RC-NILS). Five insertion events of a construct employing *zagl 1* driven by an actin promoter in a maize inbred background showed significant effects on ear length, ear diameter, and number of kernels per row. A RNA interference construct driven by an ubiquitin promoter was assessed in thirteen independent insertion events each in two different inbred maize backgrounds and showed significant effects on multiple ear traits as well as plant height and days to anthesis. Finally, to differentiate the phenotypic effects of the maize and teosinte alleles, RC-NILs were developed for *zagl 1* from heterogeneous inbred families taken from a BC2S3 population between *zea mays* ssp. *parviglumis* and the maize inbred line W22 as the recurrent parent. Lines with the teosinte allele flowered significantly earlier ($p > 0.001$) by approximately one day. Although a shattering phenotype was not observed in an inbred maize background, *zagl 1* has pleiotropic effects on flowering time, ear morphology, and plant architecture traits that are likely targets of selection during domestication.

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P241

Integrating Biological Function Prediction, Rare Alleles Testing, and Functional Network Screening into Association Mapping for Flowering Time in Arabidopsis

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The high-density array-based genome-wide association studies (GWAS) are being complemented with the whole genome exome sequencing and the whole genome resequencing-based association studies. Here we presented a composite resequencing-based genome-wide association study (CR-GWAS) strategy to systematically exploit the collective biological information and analytical tools for a robust analysis. We showcased the utility of this strategy by using an Arabidopsis resequencing data as an example. With CR-GWAS, bioinformatic predictions of biological function alteration at each locus were integrated into the process of association testing of both common and rare variants for complex traits with a suite of specialized statistics. Significant signals were then filtered with a priori candidate loci generated from genome database and gene network model to obtain a posteriori candidate loci. With the proposed strategy, we confirmed the well-known true positives and identified several new promising associations. In addition, using a probabilistic gene network (AraNet) which interrogates network neighborhoods of genes, we identified AT3G03300 (*DCL2*) as a new target candidate in regulating flowering time in Arabidopsis. More importantly, our results demonstrated the potential of integrating various biological, statistical, and bioinformatic tools into complex trait dissection.

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

P242

Integrating MAGIC and NAM to enrich the genetic network underlying the maize immune response

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This project seeks to expand and enrich the genetic architecture of the HR (hypersensitive response), the plant kingdom's most important immune response. However, instead of using artificially induced variation to do so, we are making use of the variation that is present naturally in the maize germplasm. Our rationale for this derives from the fact that, despite the use of exhaustive mutagenesis screens in many plant species, our understanding of how HR is triggered and executed remains incomplete. One way forward is to exploit natural variation, which has been generated and selected over millions of years of evolution. However, a major challenge to this approach is how to sift through the enormous diversity available. To this end, we have devised a simple yet effective method to discover and characterize useful alleles. This method, a variation on enhancer/suppressor screening that we have called MAGIC (for Mutant-Assisted Gene Identification and Characterization), makes use of the phenotype of a mutant (for a gene affecting the trait of interest) as a reporter to discover and analyze relevant, interacting genes present naturally in diverse germplasm. Using a constitutively-active (semi-dominant) allele of the of the Rp1 disease resistance gene in a MAGIC screen of the diversity panel, an amazing amount of variation capable of enhancing or suppressing the HR response was revealed in the maize germplasm. Since B73 had a moderately suppressing effect on HR and Mo17 enhancing, it allowed us to conduct a MAGIC screen on the IBM RILs to uncover a major QTL. We have named it Hrml1 (HR modulating locus-1). Now we are integrating MAGIC with NAM not only to uncover additional Hrml loci but also to clone the genes underlying them. The identity of the genes/QTL thus identified will be confirmed by a combination of targeted EMS mutagenesis and/or transposon tagging techniques.

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P243

Investigating the genetic basis for naturally occurring primary root growth variation in *Zea mays*

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Root growth rates are highly sensitive to environmental perturbation and exhibit continuous genetic variation, making it difficult to partition the variance to causative loci. To investigate the genetic basis of plant root growth variation, we examined 206 inbred lines from the intermated B73xMo17 (IBM) population. B73 primary roots grew faster than Mo17 primary roots in control, osmotic stress, and recovery conditions. We identified nine quantitative trait loci that influence primary root growth which explain 3-15% of the variance. We introgressed a B73 allele from chromosome 1 into Mo17 to confirm the effects of the locus. This introgressed segment, Mo17-B1.03, enhanced root growth in control conditions and increased the proportion of explained variance to 66%. To investigate the molecular basis for the Mo17-B1.03 phenotype, we identified genes differentially expressed between Mo17-B1.03 and Mo17 without the introgressed segment, Mo17-M1.03, in control and osmotic stress conditions. A number of genes were found to be differentially expressed between 1) Mo17-B1.03 and Mo17-M1.03 and 2) control and osmotic stress conditions. Genes associated with oxidative stress were differentially expressed between Mo17-B1.03 and Mo17-M1.03 as well as between control and osmotic stress conditions suggesting a role for free radical scavenging in explaining genetic diversity for root growth.

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P244

Joint Linkage-Association Mapping of Maize Height and Related Traits

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Plant height is a classic quantitative trait, highly heritable, easily measured, and a principal determinant of light capture, carbon sequestration, and nutrient allocation. By joint-linkage association mapping in the Nested Association Mapping population (NAM), we have dissected the additive genetic variance in plant height across 27 diverse maize inbreds. Several correlated traits including ear height, node number, days to silk, and days to anthesis, as well as derived traits such as average internode length, growth rate, and primary ear placement were also mapped to assess their pleiotropy with height at the genetic level. The resolution of our analyses was greatly enhanced by recent sequencing of a Second Generation Maize Hapmap as well as advancements in QTN mapping procedures and will be discussed. Significant associations near several candidate genes in pathways known to influence apical growth were identified. Validation of our results is underway through examination of near isogenic lines (NILs) possessing complementary tropical introgressions (CML277, CML333 in a B73 background) on chromosome 9L. Despite tropicalness of the donors, no pleiotropy with flowering was identified at the plant height loci by joint-linkage association mapping or comparison of the NILs.

Funding acknowledgement: United States Department of Agriculture (USDA)

P245

Kernel weight and composition quantitative trait loci (QTL) from single-kernel analysis

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The composition of maize kernels is an important trait for US corn producers. The typical mature maize kernel is composed of 70% starch, 14% protein, and 4% oil with 10% residual components. The endosperm contains the vast majority of total kernel starch and protein, while the embryo contains greater than 85% of kernel oil. Breeders and geneticist have leveraged natural variation to select for improved quality and have mapped genetic loci that impact kernel composition. However, in large part, the genetic determinants of kernel composition remain unknown. In this study, the Intermated B73 Mo17-Recombinant Inbred Line (IBM-RIL) mapping population was used to identify genetic loci involved in kernel weight and composition. The IBM population was grown six times between 2006 and 2010 across diverse environments including Iowa, Wisconsin, Hawaii, and Florida. To date, approximately 75,000-80,000 kernels from these populations have been analyzed by our single kernel near-infrared (NIR) spectroscopy platform, which collects individual kernel weights and NIR spectra. The relative levels of kernel components are determined from the spectral data using partial least squares (PLS) regression models. Analysis of 5 of the 6 environmental replicates indicates that between-population trait correlation coefficients are highest within Florida replicates, but significant correlations were also observed for other environmental replicates for multiple traits. Single marker regression suggests several QTL for kernel weight and kernel oil content overlap between environmental replicates, but the majority of QTL detected in each replicate were not stable in the different environments. We conclude that there is significant gene by environment interaction for kernel weight and composition traits.

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P246

Miami White Corn: Genetic Relationships Between Native American Landraces

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Native American maize landraces are culturally important and represent collections of genetic diversity that are threatened by introgression from modern maize cultivars. Miami White Corn (MWC) is a flour corn that was historically recorded as a unique landrace belonging to the Miami Tribe (Trowbridge 1938), but its cultivation was abandoned in the 1950's with the advent of modern hybrid maize. Fortuitously, MWC was rediscovered in 1995, and is currently grown by multiple tribal members in Indiana, Ohio, and Oklahoma. However, little is known about the relationships of MWC to other maize cultivars or the degree to which introgression from other maize in the landscape is affecting the genetic integrity of MWC. Microsatellite analysis is being undertaken to estimate the relationships of MWC, using publically available datasets (e.g. Vigouroux et al 2008). We will use microsatellite analysis and/or the Maize SNP chip to explore the genetic coherence of Miami White Corn, in order to provide a knowledge base that will help in preserving the remaining genetic diversity and cultural value of this landrace maize.

P247

Maize Domestication and Genetic Factors for Seedling Emergence

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Maize (*Zea mays* ssp. *mays*) was domesticated from teosinte (*Zea mays* ssp. *parviglumis*) in a single domestication event approximately 9,000 years ago in central Mexico. During domestication, maize experienced a domestication bottleneck, direct and indirect selection, and genetic drift. These genetic forces have caused a reduction in allelic variation across the whole genome and additional reduction at potentially important loci in modern maize. The analysis of domestication traits, such as seedling emergence and vigor, in a diverse genetic background including diversity from teosinte will provide evidence for specific genes important for domestication. Flint-Garcia, USDA-ARS, developed a BC_4 double haploid population of 88 teosinte introgression near isogenic lines (NILs) for analysis of genetic characteristics in a B73 background. The set of NILs were analyzed for three seedling emergence traits; warm germination (WG), rate-of-emergence vigor (REV), and infected soil cold germination (ISC). Seed for analysis was produced in two environments, Columbia, Missouri, 2008 and Madison, Wisconsin, 2010. The genotypes were evaluated in a randomized complete block design with two, three, and four replicates per trait, respectively. The correlations between the REV days-to-emergence and the other two traits were not significant ($p>0.05$). The correlation between the REV final stand and ISC was significant at 0.66 ($p<0.01$) and REV final stand with the WG was also significant at 0.55 ($p<0.01$). The WG and ISC correlation was 0.59 ($p<0.01$). Of the 88 genotypes in the population analyzed from the 2008 data, there was one entry significantly different ($p<0.05$) for all tests, and five that were significantly different for the three final stand traits. The REV had nine entries with significantly longer average days-to-emergence and four of those entries were significant for at least one of the other traits ($p<0.05$). Genomic association mapping with the seedling phenotypes is currently underway. Analysis with four other teosinte-NIL populations will be conducted to verify the results.

Funding acknowledgement: United States Department of Agriculture (USDA), University of Wisconsin-Madison, Monsanto

P248

Maize interploidy hybridization barrier: a quantitative approach

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In maize, crosses between plants of different ploidy typically result in failed seeds with the endosperm manifesting the first evidence of aberrant development. These crosses highlight the roles of both parent-of-origin and gene dosage in the proper development of plant seeds. When the paternal genome has a larger contribution, the abnormal endosperm development that occurs is associated with a change in the cell division cycle and a failure in the accumulation of storage products. Seed lethality is prevalent. Examination of surviving seed number showed statistically significant differences between the crosses of tetraploid pollen-parents to diploid B73 versus Mo17. Crosses between tetraploid pollen parents and B73, Mo17 and B73 x Mo17 were made. Seeds were classified by mature (terminal) phenotype into five classes: surviving, viviparous, miniature loose pericarp, collapsed, and empty. These categories are consistent with failures in development occurring at sequentially earlier stages of development. B73 produces more surviving seed, while crosses to Mo17 exhibit both a greater frequency of failure and an increase in the proportion of seeds consistent with a cessation of growth early in endosperm development. No statistically significant difference was observed for seeds without storage product accumulation, referred to as collapsed seeds, or seeds that fail to enter dormancy and exhibit vivipary. By measuring allele frequency in DNA bulks from surviving offspring, QTLs affecting the interploidy hybridization barrier have been found in crosses between F1 B73 x Mo17 and tetraploid maize. To dissect genetic control of aberrant seed development in interploidy crosses, the intermated B73 x Mo17 (IBM) population was crossed with a tetraploid pollen parent. A QTL examination of these data is underway to determine genes involved in the maize hybridization barrier.

Funding acknowledgement: Agricultural Research Programs-Purdue University

P249

Mapping and characterization of a flowering time mutant in Zea mays L.

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Flowering time is a key trait for the adaptation of maize to its environment. From an ethyl methane sulphonate mutagenized B73 population, we identified a true-breeding mutant line B73mut that transitions early from the vegetative developmental stage to the generative developmental stage. The mutant plants shed pollen one to three days earlier, develop fewer nodes, and are shorter than wild type B73 plants. To identify the genetic basis for the mutant, we hypothesized 1) that F2 mapping populations derived from the mutant crossed with two different inbred genotypes would segregate for a common QTL, with the B73mut allele conferring earliness, and 2) that the QTL would not have been detected in prior studies with the same or related parents. To test these hypotheses, we assayed two F2 populations, B73mut X MO17 and B73mut x A619, each with 160 individuals, for days to pollen shed, plant height, and plant node number. Populations were assayed with 100 single nucleotide polymorphism markers spread across the 10 maize chromosomes. We found a number of highly significant loci for all three traits. One QTL on chromosome 8 was shared between both crosses. However the B73mut derived allele delayed flowering, and this QTL was previously identified in B73 x MO17 mapping population. We are currently examining a large mapping population to improve our power to detect shared QTL within both populations.

P250

Model building in support of computer simulation to guide key decisions in plant breeding

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A number of crucial decisions face the plant breeder before any field activities directed to crop improvement are actually initiated, primarily related to 1) choice of germplasm, 2) the target set of environments for which an improved cultivar will provide fit, and 3) breeding strategy options. Because of the impact and modern complexity of these decisions and the diversity of options brought by scientific advancements, computer simulation can be an important resource for the breeder to inform and guide choices if based on effective models. Models must effectively represent the way the genome functions in the species of interest and the process of seed product development, and incorporate the analytical approach. Some principles and advancements in model building in support of computer simulation are considered and discussed.

Funding acknowledgement: funding by Monsanto Company

P251

Molecular Responses of Maize to Submergence

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Little is known regarding the physiological and molecular responses of maize photosynthetic tissue to transient (partial to complete) submergence. Recent discoveries in *Oryza sativa* L. point to a group of transcription factors, called group VII ethylene response factors (ERFs), which moderate antithetical strategies for submergence tolerance and submergence escape [Xu K., et al. 2006; Hattori Y., et al. 2009]. In *Arabidopsis thaliana*, these same group VII ERFs have been examined as key regulators of oxygen deprivation survival in plants, a stress frequently endured during flooding [Nakano T. et al., 2006; Licausi F., et al., 2010; Hinz M., et al., 2010]. Examination of the B73 maize reference genome (B73 RefGen_v2) identified thirteen group VII ERF genes. To begin to explore the molecular responses of maize photosynthetic tissue to severe inundation, plants at the four-leaf stage were subjected to complete submergence for up to three days. Height measurements revealed a limited growth response under submergence. The monitoring of mRNA levels by qRT-PCR in aerial tissues confirmed the up-regulation of three *SUB1A-like (Sbl)* ERFs (*Sbl1, Sbl2, Sbl3*) and *Alcohol Dehydrogenase1* (*Adh1*). The differential regulation of these genes in coleoptiles was also evaluated in 48h pre-germinated seeds subjected to up to three days of complete submergence. Increased accumulation of *Sbl1-3* and *Adh1* was confirmed. This study demonstrates that three *Sbl*s are co-regulated with *Adh1* in response to submergence in maize aerial tissue as well as under oxygen deprivation in coleoptiles. Further investigation is underway to determine the role these *Sbl* ERFs play in regulation of gene expression and acclimation to transient submergence.

Funding acknowledgement: National Science Foundation (NSF)

P252

Multidimensional analysis of root system architecture reveals differences in the Illinois High and Low Protein strains

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Root System Architecture (RSA) refers to the spatial organization of the root system in its growth environment, which reflects its capability to extract resources. However, the functional relationships between RSA and agricultural traits are poorly understood. Here we apply a simple yet powerful technique to image developing root systems of the Illinois protein strains [IPS] in three dimensions. The IPS lines have been selected for high (IHP) or low (ILP) seed protein content for over 100 cycles since their inception in 1896. These divergent selective pressures have had dramatic effects on the capability of IHP and ILP lines to uptake nitrogen and phosphorous from the soil, but little is known about the (presumed) accompanying changes to RSA. We document differences in the development of IHP and ILP RSA by extracting measurements from image-series and 3D reconstructions of the roots. These data are first steps toward linking key agricultural traits with a comprehensive, fine-scale view of RSA. Furthermore, this system may be used in the future as a rapid way to identify QTL in Illinois Protein Strains using existing recombinant inbred lines.

Funding acknowledgement: National Science Foundation (NSF)

P253

NDSU EarlyGEM: Increasing Genetic Diversity of Northern Corn Belt Hybrids with Tropical and Temperate Exotic Germplasms

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The NDSU EarlyGEM or Early Germplasm Enhancement of Maize Program is a long-term program to increase the genetic diversity of short-season hybrids by utilizing unique and diverse alleles not present in the genomes already sequenced (e.g., B73). Six late temperate and tropical exotic GEM breeding crosses: DKB844:S1601-507-1-B-B, CUBA117:S1520-388-1-B, FS8B (T): N1802-35-1-B-B, CHO5015:N12-123-1-B-B, SCR01:N1310-265-1-B-B and BR52051-N04-70-1 were adapted to short-seasons and incorporated via a modified backcross breeding procedure. B73, Mo17, and Iowa Stiff Stalk Synthetic (BSSS) were included in this program as checks. Useful genetic diversity present in the incorporated lines was evaluated with industry testers belonging to opposite heterotic groups across six environments in 2009 and 2010. CHO5015, BR52051, FS8B, and SCR01 derived lines produced early maturing hybrids that were competitive in yield and moisture with the top commercial checks across relative maturities. Similarly, CHO5015 and BR52051derived lines produced testcrosses with high grain protein and oil percentages when compared with top checks. DKB844 derived lines produced early testcross hybrids with not only high yield but also high extractable starch percentage as compared to top checks. Lines derived from BSSs, B73 and Mo17 did not produced hybrids with competitive grain moisture at harvest and grain quality characteristics. The results show that exotic germplasm incorporation via our EarlyGEM protocol is a new and successful way of breeding for early maturing maize. NDSU is currently the only genetic provider for these products generated from the integration of pre-breeding with cultivar development.

P254

Nature of the Genetic Variation in an Elite Maize Breeding Cross

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Maize (Zea mays L.) breeders through selection have had profound impacts on the maize genome. In this study we examine one aspect of this intense selection pressure, the extent and nature of genetic variation present in an elite maize breeding cross. Specifically genetic variation is examined with regards to genotype × environment interactions (G × E), magnitude of the genetic variance (Vg) estimates, and the underlying grain yield quantitative trait loci (QTL). Using two elite Iodent sister-lines that are 64% identical by descent, 128 recombinant inbred lines (RILs) were generated and testcrossed to a Stiff Stalk inbred line (CG102). Hybrid RILs were grown in 24 trials encompassing 4 years, 3 locations, and 3 planting densities. Additive main effects and multiplicative interaction (AMMI) analysis resolved the trials into eight unique patterns of G × E. Smaller Vg estimates were associated with the more frequently observed patterns of G × E. Nine single-effect QTL and four epistatic interactions were detected across seven of the G × E patterns; however, the single effect QTL and epistatic interactions were, in general, specific to a G × E pattern. In summary, we found extensive linkage disequilibrium (LD), reduced Vg in the more commonly occurring G × E patterns, and genetic variation due to larger effect epistatic interactions and smaller single effect QTL specific to the G × E pattern. Consequences of the genetic variation are discussed in relation to modern maize breeding programs.

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P255

Pleiotropic effects of ZmGHD7 on flowering time and inflorescence structure in maize and teosinte

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After maize was domesticated from teosinte in the Balsas river valley of Mexico, it spread throughout the Americas and became adapted to a broad range of environments. One method of adaptation was an adjustment of flowering time in response to environmental cues such as day length. While teosinte requires short days to flower, temperate varieties of maize are day neutral, i. e. able to flower in either short- or long-day environments. When grown in the long days of the temperate latitudes, teosinte takes more days to flower than in the short days of the tropics. This study focuses on a single QTL on chromosome 10 which accounts for a 7 day difference in flowering time (pollen shed) between a temperate inbred line of maize (W22) and teosinte. A set of nearly isogenic W22-teosinte lines with recombination events in the QTL region were used to fine-map the flowering-time QTL to a 0.33 Mb interval. This interval includes a single gene ZmGHD7, the maize homologue of the rice gene ghd7, which encodes a CCT domain protein. In rice, ghd7 influences flowering time, plant height, culm diameter, flag leaf length, branching in the panicle and grain yield. In order to test for similar pleiotropic effects in maize, QTL analysis for corresponding traits was carried out using the W22-teosinte recombinant nearly isogenic lines. QTL for plant height, flag leaf length, kernel row number and culm diameter co-localized with the causative region for flowering time at ZmGHD7. Since kernel row number and culm diameter were both traits under selection during domestication, the ZmGHD7 coding sequence or its 5' regulatory region may have been under selection during domestication. We found no evidence of past selection in exon 1 of the coding region. We are currently testing for selection in the 5' regulatory region.

Funding acknowledgement: National Science Foundation (NSF)

P256

Populations Derived From Elite Ex-PVP Commercial Inbreds: New Public Maize Genetics Resources

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The release of elite inbreds from plant variety protection (PVP) and utility patent protection represents an important opportunity for public maize research. Such material is aptly suited for the investigation of commercially relevant traits, heterosis, and selection. Ex-PVP inbreds have been improved for resistance to a variety of biotic and abiotic stresses, and should assist in the identification of relevant genes and useful alleles. This elite germplasm can also be incorporated into public breeding programs or serve as a genetic background for testing QTL from unimproved populations. We describe an elite diallel generated from 10 key Ex-PVP inbreds plus B73 and Mo17. The four stiff stalk synthetic and eight non-stiff stalk inbreds chosen as diallel parents are all key founders in the lineage of contemporary commercial U.S. Midwestern corn hybrids. The elite diallel has been evaluated for a large number of traits to characterize the performance of the inbreds and hybrids. An Elite Maize Association Mapping Panel of approximately 1,900 RILs generated from the 66 F1 crosses in the diallel is also presented, along with ongoing and planned experiments.

P257

Power of a non-destructive high-throughput phenotype platform in unraveling drought and salt stress related traits

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Root phenotyping is a major constraint in determining abiotic stresses in plants. Differences in root architecture and development are directly related to crop yield and abilities to withstand drought and soil-borne diseases. However, the study of crop root systems has lagged behind that of above-ground plant characteristics due to their confinement in the soil. Studying root traits often requires destructive measurements which hinders continued observations on the same plant. However, timing effects are very important to capture and understanding the genes that control e.g. drought and salt tolerance. To address these challenges we decided to develop a protocol to monitor and phenotype roots on a daily basis in a non-destructive manner using KeyGene's in house high-throughput phenotyping platform KeyTrackTM. A well characterized tomato IL library[1] was used to compare and validate results produced on the KeyTrackTM platform with respect to root and shoot growth. Roots of the ILs were successfully grown in transparent pots and imaged daily for growth and development. A successful algorithm was developed which was able to detect the roots and shoots digitally. ILs showing better root and shoot biomass were identified and chosen for detailed lead discovery to indicate genes involved in root development. Validation of the involvement in root development and drought resistance of these genes is in progress. Keygene has recently expanded its phenotyping facilities, enabling phenotyping on a much larger scale than before.

[1]Eshed, Y and Zamir, D. (1994) Introgressions from Lycopersicon pennellii can improve the soluble-solids yield of tomato hybrids. Theor Appl Genet 88:891-897.

Application for trademark registration for KeyTrack has been filed by Keygene N.V.

P258

QTL Mapping of Chloroplast and Mitochondria Genome Copy Number in Maize

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Chloroplast and mitochondria, which play important roles in photosynthesis and energy production respectively, are crucial to maize metabolism. Different from other organelles, the chloroplast and mitochondria have their own genomic DNA (cpDNA and mtDNA) beside the nucleus genome. A large amount of genes related to plant photosynthesis and energy production are distributed in these organelle genomes. Hence, the abundance of cpDNA and mtDNA may act on metabolic processes, such as carbon fixation and protein synthesis. Based on the genotyping by sequencing (GBS) protocol and nested association mapping (NAM) strategy, we are trying to map QTLs controlling cpDNA and mtDNA copy number in maize. So far, 5000 RILs of NAM panel are genotyped across whole genome using Illumina analyzer. On the basis of the genotyping data, the joint linkage result shows there are 6 and 4 QTLs mapped for the copy number of cpDNA and mtDNA, respectively. Further GWAS analysis will provide a high resolution for these QTLs.

Funding acknowledgement: National Science Foundation (NSF)

P259

QTL Mapping of Western Corn Rootworm (Diabrotica virgifera virgifera LaConte) Resistance in Maize

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During the last 60 years more than 12,000 maize accessions, comprising inbreds, synthetics, and open pollinated varieties, as well as maize relatives, like teosinte and tripsacum, have been screened for their level of resistance to western corn rootworm (WCR) larvae feeding. Less than 1% of this germplasm was selected for initiating recurrent selection programs. In general, the selected genotypes were characterized by large root systems and superior secondary root development after root damage caused by WCR larvae. However, no non-transgenic maize cultivars with high level of resistance under moderate to high insect pressure have yet been released. To overcome this problem, we are in the process of evaluating the defense response of maize to WCR feeding in a coherent framework, which includes gene expression and metabolite analyses. In addition, we investigate the genetic basis of WCR resistance in new maize materials with improved levels of resistance using linkage disequilibrium mapping approaches. Two populations each with 150 testcrossed double haploids (DHs) derived from crosses between resistant and susceptible maize inbreds were evaluated for their level of resistance in three to four different environments. For each DH testcross an average root damage score was estimated and used for QTL analysis. Appropriate LOD thresholds were determined using 1000 permutations. QTL positions were identified using composite interval mapping. Significant QTL were located on chromosomes 1, 2, 7, 8, 9, and 10. A model fitting all QTL simultaneously explained about 30% of the phenotypic variance for root damage scores in both mapping populations. How this knowledge impacts the design and efficiency of breeding programs to improve WCR resistance will be discussed. As a direct output of this project, molecular markers will be available to efficiently screen germplasm for novel defense response variants and to perform marker-assisted selection.

Funding acknowledgement: United States Department of Agriculture (USDA)

P260

QTL analysis for performance in elite Ex-PVP maize lines using a bi-parental mapping population

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Recently, elite proprietary lines developed by Pioneer Hi-Bred or Holdens Foundation Seeds, and used frequently for subsequent elite inbred production, were released from Plant Variety Protection (PVP). Because these commercial inbreds were protected, previously they could not be crossed to each other and evaluated. This represents a unique opportunity to evaluate genotypic and phenotypic variation observed in hybrids from these two different sources of germplasm. PHG35 and LH51, two important commercial germplasm lines, were crossed and F1 self-pollinated to create a mapping population consisting of 360 F2:3 lines. The mapping population was genotyped with 1,156 SNP markers and testcrossed to LH119. Grain yield was measured in the testcrosses in three environments during 2010 and combined with genotypic data for QTL analysis. The results of the initial QTL analysis will be presented. Results from this study will be used in part to develop public inbreds that, in hybrid combination, perform close to current proprietary inbreds and share similar genetic backgrounds. The experimental public hybrids will provide a hybrid platform for academic research that is more relevant to commercial germplasm.

Funding acknowledgement: United States Department of Agriculture (USDA), Dow Agrosciences

P261

QTL associated with carotene content in maize leaves

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Carotenes are present in almost all organs and plant tissues. In green leaves they are essential for photosynthesis. The biosynthesis of carotenoids occurs in the plastids, where they are synthesized via the mevalonic independent pathway, in which more than 10 enzymes and an unknown number of factors participate. A set of 200 recombinant inbred lines (RIL) of the IBM population were used to identify quantitative trait loci (QTL) and candidate genes involved in the carotenoid pathway. There are few reports about carotene QTL in maize kernels, and nothing in leaves. Therefore, the aim of the project was to measure the carotenoid content in leaves and identify QTL for lutein, β-carotene, α-carotene, β-cryptoxanthin, zeaxanthin and total of carotenoids. It is suggested that the carotenoid content and concentration in leaves depends on the specie, genotype and the plant age. In mature leaves, β-carotene represents 80% of total carotenoids, lutein 17% and cryptoxanthin 0.2%. 25 significant QTL were found on chromosomes 1, 2, 4, 5, 6, 7 and 8. A significant QTL ($P \leq 0.001$) with a high additive value was identified at bin 1.07 for lutein ($r2=0.07$), β-carotene ($r2=0.09$) and total carotene ($r2=0.1$). The genes near the significant peaks were analysed, and just one contained a known enzyme of the carotene pathway, namely fitoene synthase 2 (psy2). We conclude that in maize there are many unknown factors that affect carotenoid compound in green leaves. We postulate that these new QTLs could be non-annotated enzymes or regulatory proteins that could be further investigated with a more molecular focus.

P262

QTL-Mapping based on joint multiple populations in a Maize Testcross Experiment

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Connected multiple-line crosses have been proposed as an excellent mapping resource for QTL detection. In contrast to mapping populations derived from a single bi-parental cross, multiple-line crosses can increase the statistical power of QTL detection, estimation of the QTL location, and QTL effect. Multiple-line QTL mapping has the additional advantage that it reflects the germplasm base typically available from routine breeding programs. The aim of our study was to exploit the benefits of a combined analysis in an experimental data set of maize. The multi-cross QTL mapping dataset comprised six populations deriving from elite material crossed in a 4x4 diallel mating design. In a first step, QTL analysis was done separately in each population. In a second step, a joint-analysis over all populations was conducted, detecting several QTL for the traits grain yield and grain moisture. Our analyses revealed that multiple-cross QTL mapping is a powerful and robust method for the detection of QTL across different genetic backgrounds.

Funding acknowledgement: GABI-GAIN, German Federal Ministry of Education and Research (BMBF) within the framework of the GABI –FUTURE initiative

P263

Response of transgene and native gene expression to selection for transgene levels

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High expression of a transgene is useful for the production of high levels of transgenic protein and for the evaluation of transgenes. The goal of this research is to determine if recurrent selection for a transgene will result in higher expression, and if selection for a
transgene controlled by a native promoter will also increase expression of the native gene with the same promoter. To accomplish this goal we used transgenic maize containing a construct encoding green fluorescent protein controlled by the maize endosperm specific
27 gamma zein seed storage protein promoter. Recurrent selection was carried out in two breeding populations. After three generations of selection, the selected populations were significantly more fluorescent than control populations. Selection did not have a significant effect on the level of the 27 gamma zein. The results show that recurrent selection can be a successful way to increase expression of a transgene and that selection for a transgene
controlled by a native promoter did not have an effect on the native gene with the same promoter.

Funding acknowledgement: United States Department of Agriculture (USDA)

P264

Responses of maize (*Zea mays* L.) near isogenic lines carrying *Wsm1*, *Wsm2* and *Wsm3* to three viruses in the *Potyviridae*

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Genes on chromosomes six (*Wsm1*), three (*Wsm2*) and ten (Wsm3) in the maize (*Zea mays* L.) inbred line Pa405 control resistance to Wheat streak mosaic virus (WSMV), and genes in the same chromosomal regions control resistance to Maize dwarf mosaic virus (MDMV) and Sugarcane mosaic virus (SCMV). Near isogenic lines (NIL) carrying one or two of these genes were developed by introgressing regions of the respective chromosomes into the susceptible line Oh28, and tested for their responses to WSMV, MDMV and SCMV in the field and greenhouse. F1 progeny from NIL x Oh28 were also tested. *Wsm1*, or closely linked genes, provided resistance to all three viruses, as determined by symptom incidence and severity. *Wsm2* and *Wsm3* provided resistance to WSMV. *Wsm2* and/or *Wsm3* provided no resistance to MDMV, but significantly increased resistance in plants with one *Wsm1* allele. NIL carrying *Wsm1*, *Wsm2* and *Wsm3* had similar SCMV resistance in the field, but NIL with *Wsm2* and *Wsm3* were not resistant in the greenhouse. Addition of *Wsm2* to *Wsm1* increased SCMV resistance in the field. For all viruses, symptom incidence was higher in the greenhouse than in the field, and relative disease severity was higher in the greenhouse for WSMV and MDMV. An Italian MDMV isolate and the Ohio SCMV infected the *Wsm1* NIL, while the Ohio MDMV and Seehausen SCMV isolates did not. Our results indicate that the three genes, or closely linked loci, provide virus resistance. Resistance is influenced by interactions among the genes, the virus species, the virus isolate and the environment.

P265

Selective phenotyping to map QTL for drought and low soil-N stress tolerance in maize

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Advances in SNP technology, which have reduced the cost of genotyping much more rapidly than the cost of phenotyping, may lead to more efficient methods for simultaneously improving drought and low-N tolerance in maize. We investigated a selective phenotyping strategy where a large number of individuals is genotyped and only individuals that have undergone the most recombination events are phenotyped as a means of reducing the cost of phenotyping. Our objectives were to determine (1) optimal population sizes (N) for selective phenotyping and (2) how trait heritability affects the size of the subset to be phenotyped. Testcrosses of 238 recombinant inbreds from the intermated B73 x Mo17 mapping population were evaluated in multi-location trials in Minnesota in 2009 and 2010. Selective phenotyping increased the mean number of crossovers from 19.3 with N=238 (full dataset) to 25 with N=100 and 39.6 with N=50. Selective phenotyping was more useful for traits with moderate to low heritability (grain yield under drought conditions; h2 =0.42) than for traits with high heritability (grain moisture, h2=0.79). For grain yield under low soil-N (h2=0.49) and grain yield under non stress conditions (h2=0.54), more QTLs with larger effects were identified with the N=100 dataset than with the full dataset. With N=50, the number and effect of QTLs identified was significantly reduced regardless of heritability. We found similar trends in a barley doubled haploid population and an Arabidopsis recombinant inbred population. Our results suggest that selective phenotyping is an effective strategy for finding marker-trait associations.

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P266

Studies related to response of different growth stages of maize to waterlogging stress

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Among various abiotic stresses, waterlogging stress caused by contingent/intermittent flooding is considered to be one of the predominant factor determining the global geographic distribution of vegetation and restriction of maize yields in agriculture. Maize crops grown during Kharif (summer-rainy) season in tropics occasionally face pressures of waterlogging conditions that limits its yield potential.
Five inbreds viz., three tolerant and two susceptible were crossed in half diallel fashion to produce single cross progenies. In ESM trials, water logging treatment was given at various growth stage viz., knee high, tasseling and milky stage for 8 days, by keeping continuous submergence with an average depth of ponding of about 5 cm. After 8 days of ponding, water was drained out of the plots. Data on various traits namely, number of nodes bearing brace roots, leaf senescence, plant lodging, yield and yield contributing characters were recorded.
The results revealed that stages before 50% flowering was found to be most affected by waterlogging stress while in later stages, plants were found to develop certain adaptive traits to overcome the stress. Knee high stage was found to be most susceptible to waterlogging. Waterlogging stress severely affected various growth parameters and eventually resulted in poor kernel development and yield. Our studies suggested that maize is highly susceptible to waterlogging condition if it is exposed to the stress before tasseling, and crop susceptibility decreases gradually at later growth stages. High brace root development has been found to be related to better performance of maize germplasm under excess moisture conditions due to the role of brace root aerenchyma as an alternative source of oxygen supply under anoxic conditions. Thus, different genotypes should be screened at early stages so as to obtain tolerant genotype which can be further used in breeding programmes.

P267

Studying natural diversity on a region regulating flowering time on Chromosome 8

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A QTL controlling flowering time in a region on Chromosome 8 was identified in studies using the Nested Association Mapping population (NAM). Two QTLs already described, *Vgt1* and *Vgt2*, could be contributing to the allelic series observed for the NAM QTL. We are examining the region to determine if the different effects at this QTL are the consequence of an allelic series at one locus, or different loci with different effects for each loci, or a combination of both (allelic series for different loci). We are evaluating polymorphisms responsible for the regulation of flowering time – other than the known MITE— on a 15 Mb region including *Vgt1* (*ZmRap2.7*) and the candidate gene proposed for *Vgt2* (*ZCN8*). We will present: (1) results using nearly million markers for the region in hundreds of maize and teosinte inbred lines; (2) advances in the efforts to dissect the QTL through a set of different families of NILs representing both early and late backgrounds; and (3) results of the genome-wide association study (GWAS) using NAM. Our study suggests this interesting region includes several mechanisms involved in the regulation of flowering time that have been selected at different moments during the domestication and diversification of maize.

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P268

The Doubled Haploid Facility at Iowa State University

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The development of homozygous lines is an important, but time-consuming process in plant breeding and research. The in vivo induction and subsequent doubling of haploids is an efficient alternative to generate homozygous offspring in two generations. The Doubled Haploid Facility (DHF), established in 2010, provides haploid doubling for maize to ISU scientists, as well as off-campus academic researchers and breeders. The mission of DHF is to provide expertise and service in the production of doubled haploid lines in maize, to improve the technology in order to get higher success rates and lower costs, and to teach and train scientists and students (http://www.plantbreeding.iastate.edu/DHF/DHF.htm). Our research objectives are (1) marker development for induction ability, (2) development of inducer lines adapted to different environments, (3) development of technologies for mechanical pre-sorting in haploid selection, (4) introduction of additional selective marker for haploid selection, and (5) finding alternatives to colchicine for chromosome doubling. During our first year, research focused on the comparison of different application methods of colchicine for chromosome doubling.

Funding acknowledgement: Department of Agronomy - Iowa State University

P269

The Effect of Intermating on Hybrid Trait Variation and QTL Mapping in Maize (*Zea mays* L.)

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The effect of intermating on hybrid trait variation and QTL mapping was studied within a population developed from two phenotypically similar parents. Two populations, a selfed recombinant inbred line (SRIL) and an intermated recombinant inbred line (IRIL), were developed from the same parental inbred lines, the short season Iodent inbred line CG60 and the short season Stiff Stalk inbred line CG102. Plants within these populations were crossed to an inbred line from a different heterotic group, LH295 of the Lancaster Sure Crop heterotic group, and resulting testcross individuals were evaluated for grain yield and a number of agronomic traits in six environments. Genetic variance for these traits did not significantly differ between the IRIL and SRIL populations. In addition, heritability was similar in the SRIL and IRIL populations, and correlations between traits did not differ significantly. Thus, our results indicate that alleles that contribute to phenotypic variation are not in repulsion phase linkage. We also identified 12 QTL in the SRIL population and 6 QTL in the IRIL population. The QTL confidence intervals within the IRIL population were reduced by a factor of 1.65 compared to SRIL. However, few QTL were shared between the IRIL and SRIL populations, suggesting that a number of small effect loci segregate within these populations.

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P270

The Effect of Photoperiod Sensitivity and Variation in Nitrogen Utilization on Total Biomass Accumulation in Corn (Zea mays)

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Rising concerns regarding global energy consumption and environmental changes have increased interest in biomass accumulation from both food and dedicated bioenergy crops. The phenotypic diversity of maize offers flexibility in growing as a crop for both grain and biofuel feedstock production. The use of photoperiod sensitive germplasm, which delays flowering and lengthens vegetative growth, has been suggested as a possible method to increase total biomass, but little is known regarding the genetic basis and phenotypic expression of this trait in many species. Here we measured the grain and stover biomass of corn (*Zea mays*) overexpressing *Glossy15*, a gene involved in shoot maturation, which has previously been observed to delay flowering time in a manner similar to photoperiod-sensitive cultivars. In comparison, we also measured total grain and stover biomass for hybrids representing diverse temperate, temperate x tropical and tropical x tropical genetic backgrounds. Our results suggest that manipulation of shoot maturation time via *Glossy15* overexpression produces phenotypic changes comparable to those observed in photoperiod-sensitive tropical germplasm. Shifts in assimilate partitioning also significantly influence nitrogen utilization for both grain yield and total biomass production. These findings suggest that significant increases in sustainable total biomass production via single-gene changes is achievable in maize and related C4 grass species.

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P271

Mutator-like elements with tandem TIRs

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Mutator-like elements (MULEs) are widespread in plants and most of them have long Terminal Inverted Repeats (TIRs), which distinguish them from other DNA transposons. Pack-MULEs refer to a subgroup of *Mutators* and MULEs that capture gene fragments. It is known that the long TIRs of *Mutator* elements harbor transposase binding sites and promoters for transcription. This implies that TIR sequence is critical for transposition and for expression of sequences between TIRs. Here we report the presence of *Mutator* and MULEs with two duplicated long TIRs located in tandem. These elements are detected in the genomes of maize, tomato, and *Arabidopsis*. Some of these elements contain gene fragments and are present in multiple copies, which suggest that the elements have been mobile. Interestingly, for some of the MULE families (based on TIR sequences), elements with duplicated TIR co-exist with elements that have single TIRs. For one of the families in tomato, the elements with duplicated TIR, which carry a putative zinc-ion binding protein, are more prevalent than their counterparts with single TIRs. The successful amplification of this particular MULE may imply either that the duplicated TIRs confer evolutionary advantage for transposition or the amplification of the internal region is beneficial to the organism.

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P272

A single gene encodes the transposition functions of *TED*, an autonomous element of the *Mutator* superfamily

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The new mutable allele *bz-m175* arose in a cross between High-Loss x High-Knob, stocks that which carry transposons from different superfamilies (1). 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 terminal inverted repeats (TIRs) and causes a 9-bp target site duplication. *TED* is predicted to encode a 774-amino-acid protein, TEDA, that is highly homologous to MURA, the *MuDR* transposase (2). 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*. Revertants were identified as fully purple kernels in *bz-m175* ears. Among 15 concordant kernels (Bz endosperm and embryo), 6 represented transposition events. Most Bz selections were nonconcordant (Bz endosperm and bz-m embryo), having arisen from megagametophytic reversions. The frequency of such events is high: >1 per 1000 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 50 stable bz-s derivatives from *bz-m175*, 11 had a *trTED* element, 29 had a defective *TED* (*dTED*) element at *bz*, the rest ten had an adjacent deletion without reinsertion of *TED*. 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 transposons, and transposes to unlinked sites, like *MuDR*.

P273

Active MuDR elements modify but do not heritably reactivate silenced MuDR elements in maize

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We are using a simplified genetic system to study the interaction between active and silenced MuDR elements. When a line carrying the MuDR inverted repeat derivative Mu killer (Muk) is crossed to a line carrying a single active copy of MuDR, the MuDR element is heritably silenced. When Muk segregates away, extensive methylation persists in the region corresponding to the transposase binding site as well as in the region containing the transcriptional initiation sites. Upon reintroduction of an active element, methylation around the transposase binding site in the silenced element is greatly diminished, whereas methylation in the transcriptional initiation region persists. The loss of methylation around the transposase binding site is likely due to protection by the transposase, since methylation of the same region is also observed in a non-autonomous element in the absence of the transposase. Upon segregation away of the active element, methylation of the transposase binding region of silenced MuDR elements is re-established and the element remains inactive. We also demonstrate that a transcriptionally active deletion derivative of MuDR that encodes non-functional transposase is heavily methylated in the transposase binding site region, supporting the idea that transposase binding prevents default methylation from occurring and demonstrating that this default methylation is not involved in transcriptional silencing. Together, our data suggest that default modification at the transposase binding site and methylation at the transcriptional start site are quite distinct processes, despite the fact that they both involve DNA methylation and require small RNAs.

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P274

Analysis of *Ac/Ds* transposons in the maize inbred lines B73 and W22

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Ac and *Ds*, the first transposons ever described, have become a valuable gene knockout resource in maize. *Ds* elements were operationally defined by McClintock as nonautonomous elements that transpose in the presence of the autonomous element *Ac*. They comprise a heterogeneous group of transposons that share the 11-bp terminal inverted repeats (TIRs) and at least some of the subterminal oligomeric repeats where the *Ac* transposase binds, but can vary greatly in size and composition. The three classes of *Ds* elements identified so far are *Ds-del*, internal deletion derivatives of *Ac*; *Ds1*, 400-500 bp in size and sharing little inner sequence homology with *Ac*, and *Ds2*, variably-sized elements containing about 1 kb of sequences from the *Ac* termini and internal sequences unrelated to *Ac*.

We have analyzed the entire complement of *Ds*-related sequences in the genome of B73, a maize inbred that lacks *Ac*. We restricted our analysis to elements that possessed *Ac/Ds* 11-bp TIRs and were flanked by 8-bp target site duplications and identified 903 such elements. The three largest elements found, ranging in size from 4.2 to 5.1 kb, were closely related to *Ac*, but carried either internal deletions or duplications. All other elements were much shorter, once extraneous insertions were removed. There are 331 *Ds1* elements and 39 *Ds2* elements in B73, some of which are likely mobilized by *Ac*. Two new classes of *Ds-like (Dsl)* elements were identified. *Dsl3* elements lack extensive subterminal homology with Ac, but carry fragments from various parts of the Ac transposase gene. There are 44 such elements in B73. *Dsl4* elements share little similarity with *Ac* outside of the 11-bp TIR and have a modal length of ~1 kb. The majority of *Ds*-related elements in B73 (486/903) fall in this class. None of the *Dsl4* elements tested responded to *Ac*. *Ds*-related elements occur in all ten chromosomes, in numbers proportional to chromosomal length.

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P275

Characterizing histone modification dynamics in maize pericentromeres

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We are interested in how pericentromeric marks such as histone methylation are heritably maintained, and we are looking for modifications of the N-terminal tail of Histone 3 that can be used to differentiate and define the functional pericentromeric regions. In maize, pericentromeres are apparent as vast domains adjacent to the centromeres on the physical map that are nearly invisible on the crossover map[1]. Using *in situ* hybridization, we have previously shown that a particular chromatin modification, such as H3K27me2, is highly enriched at pericentromeres[2]. We are now using a combination of chromatin immunoprecipitation and transposon display (ChIP-TD) on a defined set of Cinful and Zeon transposon insertion sites we have identified on chromosome 2 in order to further characterize both H3K27me2 and additional pericentromere chromatin modifications. We are preparing to use the same set of loci and ChIP-TD on a collection of lines that were derived from B73 or Mo17 and have been separated for at least ten generations. Our hypothesis is that histone marks diverge more rapidly than DNA sequence, and that the stability of the histone marks will vary with respect to where Cinful and Zeon elements are relative to centromeres. Our study will allow us both to identify epigenetic marks that are prevalent in pericentromeric regions, and to understand their dynamics relative to centromeres.

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P276

Cloning *ufo1* through transposon tagging

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Unstable factor for orange1 (*ufo1*) has been shown to play a role in maintaining epigenetic marks in the maize genome. The *Pericarp color1* (*P1*) gene has over 100 known alleles resulting in different pigmentation patterns of the pericarp and cob glumes. In the presence of a dominant *Ufo1-1* mutation the normal pattern of a *P1* allele breaks down resulting in increased pigmentation of the pericarp, cob glumes, and even various tissues on the plant body. It has been shown that this breakdown in expression pattern caused by *Ufo1-1* is also associated with changes in the epigenetic state of the *P1* locus. To further understand the function and nature of the *ufo1* gene it must be cloned. Efforts to clone the gene are underway using transposon tagging. Transposon tagging has become a viable tool for gene identification using the AC/Ds library created by the Brutenell lab. This library contains Ds elements distributed evenly at 8-12 cM throughout the maize genome for use in regional mutagenesis and trasnposon tagging. *Ufo1-1* has been mapped to chromosome 10.3. To increase the chances of a Ds insertion three different Ds insertion lines are being used: ctg 395, ctg 399, and ctg 401. *Ufo1-1* is a dominant mutation but has been shown to have poor penetrance. This will make identifying transposon insertions difficult by phenotype alone. Recently a pair of SSR markers were found to be in tight linkage with *Ufo1-1* allowing a tentative genotyping to be made which will enhance efforts to tag and clone the gene.

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P277

Diversity and abundance of Mutator transposable elements in maize and teosinte inbreds: New insights into their evolution and behavior

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Mutator (Mu) transposable elements comprise one of the most active mutagenic systems in plant biology, introducing natural variation as well as providing a powerful genetic tool. Through a combination of bioinformatic analyses and Mu-flank sequencing, Mu transposon copy number and positioning was analyzed for three common maize inbreds (B73, Mo17, and W22) and five teosinte inbred lines. Data demonstrate greater-than-expected numbers of Mu elements in the inbred lines tested, with an average of around 200 Mu inserts per maize inbred, and highlight the diversity within the Mu classes and among both maize and teosinte inbreds. In addition, phylogenetic comparisons of the 180 bioinformatically-identified Mu elements in B73 show the diversity and relatedness of the diverse Mu classes, and reveal new aspects of Mu transposon evolution and behavior. Examination of the internal regions of Mu's in the B73 genome identified multiple transposase-related sequences. Several of these were highly similar to, but distinct from, the autonomous Mutator element, MuDR, and may indicate a greater level of complexity in the regulation and activity of the Mu transposons than MuDR-dependent transposition alone. The Mu12 class of elements (first noted by Dietrich et al. 2002, and expanded here) were more numerous and diverse than the entirety of other known Mu elements (the canonical Mu elements Mu1-Mu9, plus Mu10 and Mu11 elements). These findings raise questions about the contribution of Mu12 elements to genome variation within teosinte and maize, as well as their potential utility in mutagenic resources such as the UniformMu population.

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P278

Does Transposition-Mediated DNA Re-Replication Generate Segmental Duplications?

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Every DNA segment in a eukaryotic genome normally replicates once and only once per cell cycle to maintain genome stability. Our recent results revealed that this restriction can be bypassed through alternative transposition, a transposition reaction in which a pair of *Ac* termini from two different transposons is involved. The transposons and their flanking sequences can replicate twice in a single cell cycle if the excised *Ac* ends insert into an unreplicated site. Our model suggests that the second round of DNA replication can spontaneously abort to generate Double-Strand Breaks (DSB) which can be repaired by either Homologous Recombination (HR) or Non-Homologous End Joining (NHEJ). In some cases, re-replication followed by repair may generate segmental duplications at the transposon reinsertion site. These results show how alternative transposition coupled with DNA replication and repair can significantly alter genome structure and may have contributed to rapid genome evolution in maize.

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P279

Environmental stress-induced epiallele formation and inheritance in Zea mays: a multiple approach

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In the last years it has became evident that epigenetic marks such as DNA methylation, histone modifications and histone variants play a primary role in creating alternative states of gene expression. The formation of epialleles that can be propagated mitotically and, in some instances, transmitted to the progeny remaining stable for several generations has been well documented in plants. In particular, environmental cues are thought to activate specific epigenetic mechanisms, which add epigenetic marks and in consequence alter patterns of gene expression, destabilize the plant genome and cause phenotypic changes. In this respect, environmentally triggered formation of epialleles and their maintenance represent an important, yet unexplored, source of variation and adaptive power that can contribute to improvement of crop plants.

In the framework of the FP7 European project entitled AENEAS (Acquired Environmental Epigenetics Advances: from Arabidopsis to maize) which aims to "explore" environmentally-induced epigenetic changes as the "new frontier" of natural and artificial variability, we are investigating the detailed mechanisms of epiallele formation in response to environmental cues and their heritable maintenance in maize.

Reproducible protocols for temperature shift treatments, salinity and drought stresses have been optimized to induce epigenetic changes in maize.

In parallel we are analyzing at genome-wide level the effects of cold stress on DNA methylation profiles coupling bisulfite conversion of unmethylated cytosines with Illumina sequencing (BIS-Seq). Epigenetic regulation of gene expression in response to cold stresses will be also analyzed by mRNA-, miRNA- and CHIP-Seq. All together the results obtained by these different approaches will allow to identify a robust list of sequences target of epigenetic regulation (epitargets) belonging to three main epigenetic pathways (autonomous, small RNA and CpG methylation). Trans-generational inheritance of these epitargets will be analyzed in stressed maize wt and mutants for the three pathways.

P280

Evidence of introgression of a maize *tb1* allele into populations of teosinte

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Domesticated maize and its progenitor, teosinte, show markedly different phenotypes, specifically with respect to branching architecture: maize is characterized by a central stalk with few to no branches while teosinte is highly branched. These morphological differences are believed to be the result of human selection during domestication and much work has gone into identifying the QTLs responsible for these differences in plant architecture. The *teosinte branched1* (*tb1*) gene, a repressor of organ growth, was identified as a major QTL involved in branching differences in maize, and further studies have shown that changes in an upstream control region lead to increased expression of *tb1* and a reduction of branching in domesticated maize. Here we use sequence and PCR genotyping of the control region and the 5' UTR of *tb1* to assess variation in a range-wide sample of wild populations of teosinte. Our results suggest that introgression from maize may play an important role in *tb1* evolution in teosinte.

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P281

Excision Frequencies of Eight Maize Ac Elements Located on the Short Arm of Chromosome 1

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The excision frequencies of eight maize Ac elements located on the short arm of chromosome 1 were analyzed and compared. These Ac stocks were provided by Tom Brutnell and are part of a larger collection of Ac elements mapped by the Brutnell laboratory in near-isogenic W22 lines (J.M. Kolkman et al. 2005, Genetics 169:981-995). For each of the Ac stocks, plants homozygous for the single Ac element and the Ds reporter r1-sc:m3 were crossed as females by a homozygous r1-sc:m3 tester W22 line. Most of the kernels on the resulting ears were heavily spotted, as expected when two copies of the Ac element are present in the aleurone. Excision events were scored as the exceptional kernels that were lightly spotted or appeared to lack spots. These kernels were removed, and using magnification, were sorted into two groups: those kernels containing at least one small spot on either the aleurone or embryo, and those without spots.

The number of ears scored for each Ac stock ranged from 16 to 77. For the 392 scoreable ears, the per ear mean values for the number of lightly spotted kernels versus nonspotted kernels was 3.90 and 3.33 respectively. Among the eight Ac stocks these values ranged from a per ear high frequency of 10.09 lightly spotted and 6.34 nonspotted kernels, to a per ear low frequency of 0.56 lightly spotted and 0.03 nonspotted kernels. These data are in agreement with the observation by Kolkman et al. that the excision frequency differs among the Ac lines. The results of the present analysis identify Ac lines that may be useful for exploring their suggestion that "it is likely that the variation in excision pattern is due to small changes in the transcriptional activity of Ac across the genome".

P282

Excision and Reinsertion of Ac Macrotransposons at the Maize p1 Locus

(submitted by Dafang Wang <dwang@iastate.edu>)
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We identified three macrotransposons at the maize p1 locus: each macrotransposon is composed of a segment of maize p1 genomic DNA (up to 15 kb) bounded by a fractured Ac element (fAc, 2039 bp 3' portion of Ac), and a full-length Ac element in direct orientation. The resulting macrotransposons are of 16, 16.5 and 22 kb total length. Candidate macrotransposition events were initially identified by loss of p1 function, and further screened by diagnostic PCR. Among 184 candidates screened, we identified 15 cases of macrotransposon excision and reinsertion elsewhere in the genome. The reinsertion sites were cloned (by Ac casting or inverse PCR) for seven cases, and target site duplications were identified in five of seven cases. Our results show that Ac macrotransposons are capable of mobilizing a typical full-length plant gene and thus may have contributed to the erosion of gene co-linearity in syntenic regions during plant genome evolution.

Funding acknowledgement: National Science Foundation (NSF)

P283

Genomic characterization for parasitic weeds of the genus *Striga* by sample sequence analysis

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Sample sequence analysis (SSA) on randomly selected clones can describe the basic properties of complex plant genomes with a small data set and SSA-adapted annotation procedures. Phylogenetic analysis employing six chloroplast loci indicated the ancestral relationships of the five most agriculturally-important *Striga* species, with the unexpected result that the one legume parasite (*S. gesnerioides*) was found to be more closely related to some of the grass parasites than many of the grass parasites are to each other. Generation of ~2200 reads each for seven *Striga* DNA samples allowed identification of the highly repetitive DNA content in these genomes. Genome sizes were determined by flow sorting, and the values of 615 Mb (*S. asiatica*), 1227 Mb (*S. gesnerioides*), 1425 Mb (*S. hermonthica*) and 2460 Mb (*S. forbesii*) suggest a ploidy series. The fourteen most abundant repeats in these *Striga* species were identified and partially assembled. Annotation indicated that they represent eight long terminal repeat (LTR) retrotransposon families, three tandem satellite repeats, one LINE retroelement, and one DNA transposon. These repeats were differentially abundant in each species, with the LTR retrotransposons and the satellites most responsible for variation in genome size. A phylogenetic analysis of the retroelements suggests all of these repeats are most closely related to repetitive DNAs in other closely-related plants, and are not products of horizontal transfer from their host species. Each species had some repetitive elements that were more abundant and some less abundant than the other *Striga* species examined, indicating that no single element or any unilateral growth or decrease trend in genome behavior was responsible for variation in genome size and composition.

Funding acknowledgement: National Science Foundation (NSF), Fulbright Foundation, the Guggenheim Foundation, the Georgia Research Alliance, and the Giles Professorship

P284

Identification of imprinting genes in maize endosperm through transcriptome sequencing

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Genetic imprinting is known to play important roles in seed development of higher plants, but its exact extent and regulation is unknown due to the scarcity of confirmed imprinted loci. Upon sequencing of the transcriptomes of endosperms of reciprocal hybrids, we have found that hundreds of genes in maize endosperm are subjected to genetic imprinting. We also identified a number of imprinted non-coding RNAs that are transcribed from intergenic, intronic and promoter-associated regions of protein coding genes. Imprinted genes identified show a tendency of forming clusters. The details of our imprinting screening process and the annotation of identified imprinting genes will be presented in the meeting.

Funding acknowledgement: 973 program, 863 project, National Natural Science Foundation of China

P285

Influence of genetic background on *Ac/Ds* transposition activity

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We are involved in a project to identify gametophyte specific mutations that result from transposed *Activator* (*Ac*) transposable elements. The advantage of using *Ac* is that it can act both as a genetic and molecular marker, which facilitates analysis of mutant alleles. A disadvantage is that *Ac* tends to transpose to loci that are tightly linked to the donor locus. This problem is being addressed by using multiple stocks such as those developed by the Brutnell Laboratory at the Boyce Thompson Institute, where each stock has a donor *Ac* located at a different chromosomal location. Another disadvantage of *Ac* is that this element transposes at relatively low rates. It has long been known that *Ac* activity is influenced by genetic background and all the *Ac* donor stocks are in a W22 background. We were interested to see how *Ac* activity is affected in the F1 when the *Ac* donor stock is crossed with various other inbreds. Three different donor *Ac* stocks (*mon00108, mon00178, bti03525*; all in W22) were crossed with five different non-*Ac* stocks (4Co63, GEMS0067, H99, L289 and W23). Instead of assaying *Ac* transposition directly, we looked at the trans-effect that the *Ac*s had upon the non-autonomous *Dissociation* (*Ds*) element that is responsible for the mutability of the *r1-m3* allele. Specifically we counted the number of stable-colored kernels produced on F1 ears. For simple counts of stable-colored kernels, the W23/W22 F1s consistently yielded the highest rate among all three *Ac* donors. Adjacent colored kernels were then grouped into multiple-kernel sectors with the assumption that each sector was likely to have been the result of a single transposition earlier in development. In that case H99/W22 had the highest rates with *mon00108* and *mon00178*. These results indicate that genetic background differentially affects timing of *Ac* transposition as well as overall activity.

Funding acknowledgement: National Science Foundation (NSF)

P286

Maintenance of tissue-specific gene silencing and paramutation is associated with histone modification regulated by *unstable factor for orange1* (*ufo1*) in maize

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Tissue-specific gene silencing and paramutation in plants require epigenetic mechanisms that affect transcription. However, it is still not clear whether a shared mechanism is involved in gene silencing and paramutation. Here, we report *unstable factor for orange1* (*ufo1*) that plays a role in maintenance of the silenced state for these two cases of suppression. The *Ufo1-1* mutant has been characterized as a dominant trans-acting modifier of *pericarp color1* (*p1*) gene. The *p1* gene encodes a Myb transcription factor and regulates the accumulation of phlobaphenes (flavonoid pigment) in floral organs. *P1-wr* (white pericarp and red cob glume) allele of *p1* contains a multicopy gene structure and its DNA hypermethylation is correlated with tissue-specific pigmentation. *P1-wr* does not participate in paramutation and thus provides model system to study tissie-specific silencing. We have previously demonstrated that presence of *Ufo1-1* leads to DNA hypomethylation at *P1-wr*, leading to enhanced accumulation of phlobaphenes in pericarp, cob glumes, husk, silks, leaf sheath, and tassel glumes. Using chromatin immunoprecipitation assays (ChIP), we have now identified that the wild type *ufo1* is required for maintenance of the histone marks at *P1-wr*. The repressive histone mark H3K9me2 is decreased at *p1* in *P1-wr:Ufo1-1* plants. Moreover, we also found that *ufo1* maintains the silenced state of single copy and paramutagenic allele, *P1-rr'*. Our results show that the silenced *P1-rr'* is reactivated in the presence of *Ufo1-1*, which is further correlated with decreased H3K9me2. Intriguingly, it was also revealed that H3K9me2 level at *copia* retrotransposon is affected by *Ufo1-1*. Together, our results suggest that *ufo1* may be involved in both tissue-specific silencing and paramutation. Additionally, maize *mediator of paramutation 1* (*mop1*) gene, a RNA-dependent RNA polymerase, was also reported to maintain the tissue-specific silencing and paramutation by RNA-mediated mechanisms. However, distinct from *ufo1*, *mop1* has a major effect on paramutation but a minor effect on tissue-specific silencing, while *Ufo1-1* reactivates both paramutagenic and silenced allele promptly. It is not clear whether *mop1* and *ufo1* regulate gene silencing and paramutation through same pathway. Our results suggest that *ufo1* participates in tissue-specific silencing, paramutation and transposable element silencing via histone modifications.

Funding acknowledgement: National Science Foundation (NSF)

P287

Meiotically stable inheritance of natural variation for CG methylation in genetically identical maize plants

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DNA methylation patterns must be propagated at each round of cell division in order to provide the correct epigenetic information to the newly synthesised cell. However, the fidelity of this process is not absolute and methylation differences can arise between cells of the same tissue, intra-individual tissues, and inter-individuals. Different processes are known to influence the stability of the methylation patterns, including environmental cues and genetic loci features. An emerging need in epigenetic studies is to clarify at which extend genetic and epigenetic information are related. Accordingly to understand this relationship, we explored the level of meiotically stable methylation differences between plants that share an identical genome by using the methylation-sensitive amplified polymorphism (MSAP) technique. The results of this study showed that, in absence of genetic polymorphisms individual plants are characterized by meiotically inheritable methylation differences. Novel methylation variation is generated at each cycle of inbreeding and, largely, depends upon alterations of CG methylation present at gene coding regions. Additionally, we found that inheritance of methylation variation is metastable and that reversion events occurred at some loci preferentially on paternal transmission. Altogether, these data provide insights into the mechanism underlying epigenetic variations in maize.

Funding acknowledgement: Agricultural Ministery, Italy

P288

Modeling structure of Ac Transposase and Predicting Ds preferential insertion sites: a Bioinformatics approach to understanding *Ac/Ds* transposition in maize

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The maize transposable elements *Ac/Ds* constitute one of the best-characterized transposon systems in eukaryotes, yet knowledge of the *Ac/Ds* transposition mechanism at the biochemical level and of its target site preference still remain elusive. To address these two questions, we modeled the protein structure of Ac transposase and seek to predict *Ds* preferences for insertion sites using a machine learning method.

Ac/Ds is a member of the hAT transposon superfamily, in which protein structure of one member Hermes in insects has been resolved. Using a threading method with Hermes as template, we were able to model the protein structure of Ac transposase and of other hAT members. Structural alignment and functional domain/motif assessment suggest a conserved configuration at the entire structure level and highlight a more strikingly conserved structure at the DDE catalytic site. Moreover, the structure-based phylogeny, different from the sequence-based version, may better reflect the evolution of transposase functionality in the hAT superfamily.

Our group has previously shown that *Ds* has no strong target site consensus sequence, but certain DNA structural properties may influence *Ds* target site selection (Vollbrecht *et al.* 2010). Here we further examined the Nucleosome Positioning Pattern (NPP) in DNA sequences that flank *Ds* insertion sites, using existing NPP prediction tools. Preliminary results indicate a nucleosome occupancy peak centered on the insertion site, as compared to its flanking sequences. We hypothesize that nucleosome occupancy, together with DNA structural properties identified previously and potentially other features, will be useful for making genome-wide prediction of *Ds* insertion sites in maize. Insight gained from a bioinformatics perspective should add to our understanding of *Ac/Ds* transposon biology and facilitate the ease of two-component *Ac/Ds* gene tagging in maize.

Funding acknowledgement: National Science Foundation (NSF)

P289

New reverse genetics resources for maize: facile production and efficient indexing using next-generation sequencing technology

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A sequence-indexed reverse genetics resource is considered essential to fully exploit the maize genome sequence. This project will generate and sequence-index transposable element insertions using a rapid, accurate, and cost-effective method that takes advantage of multi-dimensional pooling and next-generation sequencing technologies. Specifically, the work will: (1) Sequence-index a collection of 1150 existing *Ac* transposants; (2) Complete a set of 120 roughly equidistant transgenic *Ds** launching platforms carrying easily scored markers that will 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-index 10,000 *Ds** insertion sites from model platforms using a novel method that should be generalizable to any collection of insertions produced in a common genetic background; and (4) Develop a web-searchable database of insertion site sequences cross-referenced to stocks available from the Maize Genetics Stock Center.

Using the combined excision and reinsertion markers in the construct, *C1* and *GFP*, more than 80 transgenic lines with *Ds** transposition activity have been generated. Half of the platforms have been mapped to the reference B73 genome by isolating the sequence flanking T-DNA integration sites, which oftentimes is in a genic region. More than 10,000 C' revertants bearing a *trDs** have been selected from transgenic lines with a high reversion frequency. In a test of 3,000 *C'* and *GFP* (purple, green fluorescent) selections, <0.5% were nonheritable, showing that the system will be extremely efficient for recovering mutations caused by *trDs** elements. All the target sites of these *trDs** will be mapped to the reference genome using 454-generated sequences from multi-dimensionally pooled samples.

This project will deliver a sequence-indexed reverse genetics resource, considered essential by the maize genetics community. It will produce a web-searchable maize transposant database, matching sequences of knocked-out genes with stocks that will be freely available from the Maize Stock Center. All the relevant information from this project will be accessible from MaizeGDB.

Funding acknowledgement: National Science Foundation (NSF)

P290

On the role of RNA in centromere chromatin

(submitted by Jonathan Gent <gent@uga.edu>)
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RNA can be a major determinant of chromatin structure through a variety of regulatory and structural interactions, and multiple lines of evidence have implicated roles for RNA in centromere chromatin. We are characterizing RNA derived from centromeric tandem repeats, including siRNAs, but we are focusing on a larger species of about 40 nt in length that physically interacts with the centromere chromatin. We anticipate that this RNA-centered approach, in conjunction with studies of centromere chromatin proteins, will lead to a better understanding of how centromere chromatin states are regulated and maintained.

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

P291

Profiling DNA methylation patterns in maize inbreds

(submitted by Amanda Waters <water157@umn.edu>)
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The understanding of the genetic basis for phenotypic variation within a species is advancing due to new genomics resources and technologies. However, epigenetic (heritable variation not due to sequence changes) differences can also cause phenotypic variation in plant and animal species. The prevalence, stability and specific mechanisms of natural epigenetic variation have not been well characterized. The goal of this project is to advance our knowledge of natural variation for DNA methylation in maize. We developed a custom NimbleGen 2.1M feature array with long oligonucleotide probes spaced every 200 bp throughout the maize genome and increased resolution (every 56bp) on chromosome 9. DNA methylation was then analyzed by comparing hybridization signals following meDIP (methylated DNA immunoprecipitation) relative to total genomic DNA. The protocols for assessing enrichment and identifying methylated regions will be presented. To date we have used this approach to assess methylation levels in leaf tissue of B73 and Mo17. A similar approach is currently being used to survey DNA methylation patterns across the maize Nested Association Mapping (NAM) parent lines.

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P292

Reproduction specific Argonaute genes in maize and barley and their role in transposon silencing

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RNA-mediated silencing of transposons is an important defence mechanism to suppress the proliferation of transposons in plants and animals. In plants such processes for transposon silencing have been suggested to act in both the female and male gametophytes. Argonaute proteins are key players in RNA dependent silencing mechanism and we are interested in investigating the role of female gametophyte-specific ARGONAUTEs in transposon silencing. Previously a female reproductive tissue-specific ARGONAUTE, AGO104, has been identified in maize. Transcriptional profiling of ovaries from *ago104* mutants showed an abundance of transcripts derived from transposons and repeats compared to the wild type plants, thus suggesting a female gametophytic mechanism for transposon silencing in maize. We are further studying the role of AGO4-like proteins in a large genome cereal, barley, a true diploid grass species with a genome twice the size of maize. Barley has two Ago4-like genes *Ago1002* and *Ago1003*, of which *Ago1002* shows a higher homology to *Ago104*. The comparative expression data of the barley Ago4-like gene will be presented. Mutations in the *Ago1002* and *Ago1003* genes are also being identified using a TILLING population. A comparative analysis of components of RNA-mediated silencing mechanisms may contribute to our understanding of genome expansion and larger genomes in certain species.

P293

The *rmr2* locus encodes a novel protein required for small RNA biogenesis and paramutation

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The proper ontogeny of an organism requires several layers of regulatory information in addition to the basic DNA instructions. Heritable epigenetic marks on DNA molecules and associated chromatin provide additional and reversible regulatory information to the genome and influence phenotypic readout. Paramutation describes a process in which heritable changes in epigenetic regulatory states are facilitated by trans interactions between alleles on homologous chromosomes. When present in the same individual, a weakly expressed allelic state facilitates the repression of highly expressed states. These formerly highly expressed states remain repressed and can themselves facilitate repression of a naive highly expressed state in subsequent generations. The *Rhoades* allele of the *purple plant1* locus is one such example that can exist in a repressed state (*Pl'*) that can facilitate the repression of a highly expressed state (*Pl-Rh*). Genetic screens for factors required to maintain the repressed *Pl'* state have identified several potential orthologs to components of an *Arabidopsis* RNA-directed DNA methylation (RdDM) pathway. We used *Mu*-based Illumina sequencing to identify the gene model defined by *required to maintain repression2* (*rmr2*) mutants. The *rmr2* locus encodes a novel protein, which is required for accumulation of 24 nucleotide small RNAs and for maintenance of specific cystosine methylation patterns. While *rmr2* mutants share these phenotypes with other maize RdDM-like factors, RMR2-like orthologs in multicellular plants are all currently uncharacterized. These results present novel possibilities for expanding our understanding of small RNA biogenesis and the role of these processes in facilitating paramutation.

Funding acknowledgement: National Institutes of Health (NIH), United States Department of Agriculture (USDA)

P294

The insertion polymorphism of *Mutator*-like elements (MULEs) and their influence on gene expression upon long-term selection in maize

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Mutator-like elements (MULEs) belong to a highly mutagenic and active DNA transposon family, which are widespread in plants. MULE elements have the propensity to insert into low copy and genic regions in several plant genomes studied so far, which influence the expression of adjacent genes as well as the relevant developmental processes. The Illinois Long-Term Selection Experiment (ILTSE) maize strains are elite experimental materials for a variety of studies, especially studies on the effects of selection on the kernel chemical composition (oil or protein). However, little is known about the molecular mechanism underlying the selection. Particularly, it is not clear whether transposons have played any role in the artificial selection. To this end, the insertion polymorphism of MULEs in these maize strains was studied using transposon display, a modified AFLP technique. Subsequently, the expression levels of genes with polymorphic MULE insertions were tested using RT-PCR. MULEs with similar Terminal Inverted Repeats (TIRs) are considered to be one subfamily. So far, about 30 MULE subfamilies were studied in the Illinois protein strains and more than 300 polymorphic MULE insertions were discovered and sequenced. In agreement with previous findings, ~90% of the MULE insertions are located in low-copy genomic regions (less than 3 BLASTN hits in B73 RefGen_v1) and ~30% out of which are found to be within or close to a gene (1.5 kb upstream of transcription start site to 1.5 kb downstream of transcription stop site). For subsequent studies, only those MULEs that are co-segregating with either high or low chemical strains (e.g. MULEs only present in all high protein strains but not in low protein strains) are included. Among those tested genes, about 1/3 showed reduced gene expression in maize strains with MULE insertion. The remainder of genes exhibit little or marginal changes in their expression levels, despite the presence of MULEs in their 5' regions, 3' regions or introns. So far, no significant increase in transcription level was found to be correlated with MULE insertion. Further analysis will determine whether the differential expression of genes with MULE insertions has altered the synthesis or deposition process of protein, oil, and starch in kernels of these maize strains.

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P295

Tissue Specific Alternative Splicing Expression of *Helitron*-captured Genes in Maize

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Maize *Helitrons* are known for their propensity to capture and mobilize gene fragments into different regions of the genome. The mechanism of this process and its relevance to the element or the host genome largely remain undetermined. Several mechanisms by which these elements capture gene fragments have been proposed, however, each lacks experimental evidence primarily due to the absence of *in vivo* or *in vitro* systems to assay *Helitron* activity. In some cases, *Helitron*-captured genes are transcribed giving birth to chimeric transcripts intertwining coding regions of different captured genes. These chimeric transcripts can potentially lead to the evolution of new genes containing novel domains which may serve a unique biological function. To assess the extent of this process from a genome-wide perspective, we wrote a program using Bio-Python script called *HelRaizer* (http://secs.oakland.edu/helraizer/), which uses short, conserved terminal regions to discover high-quality *Helitrons* in the maize genome. *Helitrons* detected by this program and that also exhibit +/- polymorphisms in maize inbreds and contain pieces of more than one captured gene were monitored for expression via *in silico* EST analysis. Intriguingly, expression validation of selected elements by RT-PCR analysis revealed multiple transcripts, generated by alternative selection of splice sites during pre-mRNA processing and differential expression in root and shoot tissues. These observations suggest that increase in the transcript diversity of the captured genes by alternative splicing may significantly enhance the role of *Helitrons* in creation of new genes upon evolutionary selection. It appears that *Helitrons* have a phenomenal ability to "display" new coding regions for possible selection in nature.

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P296

Transposon-Induced Chromosome Engineering in Plant Genomes

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Maize Ac/Ds transposable elements can generate a variety of genome rearrangements, including deletions, direct and inverted duplications, and translocations. These rearrangements can occur by transposition reactions that involve the termini of different copies of the Ac/Ds family: Ac 3' and 5' termini in direct orientation can undergo Sister Chromatid Transposition, leading to the formation of flanking deletions and inverted duplications (Zhang and Peterson, 1999, 2005); while Ac termini in reversed orientation can undergo transposition reactions resulting in inversions, deletions and translocations (Zhang and Peterson, 2004; Zhang et al., 2006, 2009).
We have developed transgenic systems in Arabidopsis (Krishnaswamy et al., 2008, 2010), maize (Yu et al., in preparation), and rice (Yu et al., submitted) that are capable of undergoing alternative transposition reactions. The systems utilize transgene constructs containing maize Ac termini in direct or reversed orientation. The action of Ac transposase on the Ac termini generates deletions, duplications, inversions and translocations. The current state of the project will be presented. To view an animation of the alternative transposition model, see http://jzhang.public.iastate.edu/Transposition.html.
The ability to manipulate chromosome structure in planta may provide new opportunities and approaches for functional genomics (deletions for dissection of gene clusters and complex QTLs); plant breeding (duplications to enhance agronomic traits); and novel technologies (production of minichromosomes). This work was supported by National Science Foundation Grants No. 0110170 and 0450243 to TP and JZ, and DBI 0423898 to JB.

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P297

Variation in genome-wide DNA methylation patterns among maize inbreds

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DNA methylation is an important mechanism for genomic regulation of transposable elements, repetitive sequences, and genes. Genome-wide methylation patterns of the maize inbreds B73 and Mo17 were compared using methylated DNA immunoprecipitation followed by hybridization to custom designed microarrays (meDIP-chip). The majority of methylated loci were highly conserved between the both inbreds and we frequently observe elevated methylation of near transposable elements within the B73 genome. We investigated the interaction of structural variation in the B73 and Mo17 genomes and DNA methylation. There are a number of sequences that are present within the B73 genome but absent from Mo17 while other sequences have additional copies in Mo17 relative to B73. Some of these variable copy-number sequences show high methylation levels while others are not methylated. A detailed analysis for several large regions of low genetic diversity revealed the presence of epigenetic variation in the absence of genetic variation. Differences between these two inbreds provide a glimpse of the epigenetic variability, and uniformity, found across maize.

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Late Submissions

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Genetic engineering of biofuel crops to improve renewable energy production

(submitted by Zhanyuan J. Zhang<ZhangZ@missouri.edu>)
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Genetic engineering plays a unique and important role in improving crop traits. We are developing an engineering approach to improve biofuel production as an alternative source of energy. Two of the most important crops to be engineered in our laboratory are switchgrass (*Panicum virgatum*) and sorghum (*Sorghum bicolor*). To be successful in engineering these crops, it is essential to develop an efficient *Agrobacterium*-mediated transformation process in each of these crops. The *Agrobacterium*-mediated T-DNA transfer offers several advantages over other transformation systems. However, in spite of previous reports, *Agrobacterium*-mediated transformation of these two crops has been proven to be difficult. Therefore, since our project started we have optimized a number of critical conditions affecting sorghum and particularly switchgrass transformation. These conditions included the genotypes, cocultivation temperatures and medium salt concentrations, *Agrobacterium* strains, transformation vectors, selection system and selective agents. These works have laid good foundations for improved transformation of these two crop species via *Agrobacterium*. Since improved transformation systems were well established in our laboratory, they have been used for research and transformation services. For more information on how to request switchgrass and sorghum transformation services, please contact Dr. Zhanyuan Zhang at 573-882-6922 or email: zhangzh@missouri.edu.

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