# 58th Annual Maize Genetics Conference

Program and Abstracts

## March 17 – March 20, 2016

Hyatt Regency
Jacksonville, Florida

# This conference received financial support from:

National Science Foundation
DuPont Pioneer
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KWS SAAT AG
National Corn Growers Association
BASF Plant Science
Bayer Crop Science
Dow AgroSciences
Benson Hill
NRGene
AgReliant Genetics
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***We thank these contributors for their generosity!***

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Cover image description

Shelling of variegated kernels at the green house

Cover art by


Stefania Vendramin Alegre
Florida State University

# General Information

## Meeting Registration

Thursday: 3:00 PM to 9:30 PM: Registration Office (2nd Floor Sky Bridge near Grand ballroom)
Friday: 7:00AM to 10:00 AM: Registration Office (2nd Floor Sky Bridge near Grand ballroom)

## Meals

All meals will be served buffet style in the Grand Ballroom 1-4; 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 Grand Ballroom 5-8.

Posters will be presented in the Ballroom Foyer, adjacent to the Grand Ballroom, and on the 3rd floor in the St. Johns and Clearwater rooms. Posters should be hung Thursday starting at 3 PM and stay up until Sunday morning, but must be removed by 9 AM on Sunday. During poster sessions, presenters of odd number posters are asked to stand by their posters 1:30-3:00 PM on Friday and 3:00-4:30 PM on Saturday. Presenters of even numbered posters should stand by their posters 3:00-4:30 PM on Friday and 1:30-3:00 PM on Saturday.

The maize meeting is a forum for presentation and discussion of unpublished material. **Photographing or recording of talks and posters is <u>not</u> allowed**.

## Hospitality

After the evening sessions on Thursday and Friday there will be informal socializing and poster gazing in the Grand Ballroom 1-4, with refreshments provided until 1 AM. On Saturday evening there will be informal socializing in the Grand Ballroom 1-4, with music, dancing and refreshments until 2 AM.

After 1 AM, Suite 4104, adjacent to the 4th Floor Sky Bridge is available for continued socializing. This is a "private room" for socializing and professional networking, and it is permissible for alcoholic beverages to be brought in; however, you must stay in this room if you are carrying drinks, and please dispose of all trash and bottles in the room.

## Steering Committee

Please share your suggestions and comments about the meeting with the 2016 Steering Committee

David Braun, Chair..... (braundm@missouri.edu)
Erich Grotewold, co-Chair.(grotewold.1@osu.edu)
Mark Settles, Local Host... (settles@ufl.edu)
Wes Bruce ..... (wes.bruce@basf.com)
Alain Charcosset ..... (alain.charcosset@moulon.inra.fr)
Sherry Flint-Garcia ..... (flint-garcias@missouri.edu)
Karen McGinnis ..... (mcginnis@bio.fsu.edu)
Gernot Presting ..... (gernot@hawaii.edu)
Ruairidh Sawers..... (rsawers@langebio.cinvestav.mx)
Petra Wolters..... (petra.wolters@cgr.dupont.com)
Amanda Wright..... (amanda.wright@unt.edu)
Jianbing Yan ..... (yjianbing@gmail.com)

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

## Acknowledgements

Many thanks go to John Portwood, Carson Andorf, and Mary Schaeffer for their tremendous efforts in organizing, assembling, and advertising the conference program. We also greatly thank Angela Freemyer and her team at the University of Missouri Conference Office for helping to organize the conference, handling registration and dealing with a multitude of other issues. Special thanks are also extended to Susan Wiley and the Hyatt Regency staff for their help in organizing this conference, and to Darwin Campbell and John Portwood for providing AV and other support. Thanks go to Wes Bruce, Petra Wolters, Mark Settles, and David Braun for their efforts in securing funding to support graduate student attendance at this meeting. Finally, many, many thanks go to Mark Settles for his work as local organizer and to Marty Sachs for his wisdom in all things related to the Maize Meeting.

# Useful Links

**2016 Maize Meeting Website**

http://maizegdb.org/maize_meeting/2016

**2017 Maize Meeting Website (Available November 2016)**

http://maizegdb.org/maize_meeting/2017

**Abstract Book (Electronic version)**

http://maizegdb.org/maize_meeting/abstracts/2016Program.pdf

**Cover Image**

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

# The MaGNET Program and 2016 Awards

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

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

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

## 2016 MaGNET Awardees

**Undergraduate**

| | |
|---|---|
| Mahliyah Adkins-Threats, Truman State University | Poster #159 |
| Cairo Archer, Boyce Thompson Institute for Plant Research | Poster #135 |
| Suzi Barboza-Pacheco, Oklahoma State University | |
| Merritt Burch, University of Hawaii at Hilo | |
| Asha Cotterell, Spelman College | Poster #376 |
| Stephanie Diaz, University of South Carolina – Aiken | Poster #353 |
| Morgan James, Langston University | |
| Weschester Junior, Florida A&M University | |
| Sydney Lyda, Florida A&M University | Poster #198 |
| Umnia Mahgoub, Iowa State University | Poster #117 |

**Graduate Student**

| | |
|---|---|
| Oliver Bear Don't Walk IV, Stanford University | Poster #219 |
| Israel Jimenez Luna, California State University - Los Angeles | |
| Muriel Longstaff, Brigham Young University | Poster #218 |

**Scientist**

| | |
|---|---|
| Roselyn Hatch, Purdue University | Poster #146 |

**Faculty Mentor Accompanying Student**

| | |
|---|---|
| Gokhan Hacisalihoglu, Florida A&M University | Poster #65 |
| Ramesh Katam, Florida A&M University | Poster #198 |

The MaGNET program of the Maize Genetics Conference is supported by grant IOS-1608773 from the National Science Foundation, Plant Genome Research Program.

## Schedule of Events

**Talks will be held in the Grand Ballroom 5-8.**
**Posters will be displayed in the Ballroom Foyer (adjacent to Grand Ballroom) and on the 3rd floor in the St. Johns and Clearwater rooms.**

## Thursday, March 17

| | |
|---|---|
| 2:00 PM – 6:00 PM | **OPTIONAL PRE-CONFERENCE WORKSHOPS** |
| 2:00 PM – 3:30 PM | **MaizeGDB problem solving workshop** (Daytona Room) |
| 3:30 PM – 4:30 PM | **Gramene tutorial** (Daytona Room) |
| 4:30 PM – 6:00 PM | ***Zea mays* genome assemblies** (Daytona Room) |
| | *Pre-registration recommended for the above sessions.* |
| 3:00 PM – 8:30 PM | **REGISTRATION** (2nd Floor Sky Bridge near Grand Ballroom) |
| 3:00 PM – 6:00 PM | **POSTER HANGING** (Ballroom Foyer and 3rd floor in St. Johns and Clearwater Rooms) |
| 6:00 PM – 7:00 PM | **DINNER** (Grand Ballroom 1-4) |
| 7:00 PM – 9:00 PM | **SESSION 1 – PLENARY TALKS**<br>Chair: David Braun Pages 26 & 27 |
| 7:00 PM | **WELCOME AND ANNOUNCEMENTS** (Grand Ballroom 5-8)<br>David Braun |
| 7:15 PM | **Karen Koch, University of Florida** [Plen 1]<br>*Sugar in your ears: Regulating transport, metabolism, and consequences* |
| 8:05 PM | **Jonathan Lynch, Pennsylvania State University** [Plen 2]<br>*Phenes and genes for improved water and nutrient capture in maize* |
| 9:00 PM – 10:00 PM | **REGISTRATION** (2nd Floor Sky Bridge near Grand Ballroom) |
| 9:00 PM – 1:00 AM | **INFORMAL POSTER VIEWING & HOSPITALITY**<br>(Grand Ballroom 1-4) |

## Friday, March 18

| | |
|---|---|
| 7:00 AM – 8:00 AM | **BREAKFAST** (Grand Ballroom 1-4) |
| 7:00 AM – 8:15 AM | **REGISTRATION** (2nd Floor Sky Bridge near Grand Ballroom) |
| 8:00 AM – 10:15 AM | **SESSION 2 - COMPUTATIONAL & LARGE-SCALE BIOLOGY**<br>Chair: Alain Charcosset Talks 1-6. Pages 31-36. |
| 8:00 AM | **ANNOUNCEMENTS** (Grand Ballroom 5-8)<br>David Braun |
| 8:15 AM | **Amanda Waters, University of Minnesota** [T1]<br>*Natural variation and cis-regulatory variation for response to abiotic stress in maize* |
| 8:35 AM | **Julia Bailey-Serres, University of California, Riverside** [T2]<br>*Ancient sensing and response networks regulate flooding tolerance – So what's up with maize?* |
| 8:55 AM | **Ana Paula Alonso, The Ohio State University** [T3]<br>*Deciphering kernel metabolism: how metabolomics and fluxomic can guide us* |
| 9:15 AM | **Bo Wang, Cold Spring Harbor Laboratory** [T4]<br>*Unveiling the complexity of maize transcriptome by single-molecule long-read sequencing* |
| 9:35 AM | **Linda Hanley-Bowdoin, North Carolina State University** [T5]<br>*DNA replication timing programs during development of maize root tips* |
| 9:55 AM | **Johann Joets, Institute for Agricultural Research (INRA)** [T6]<br>*Genome-wide sequence analysis of the maize FV2 inbred line provides new insights into molecular and chromosomal features of presence/absence variants and their implication in European maize genetic originality* |
| 10:15 AM | **BREAK** |
| 10:45 AM – 12:25 PM | **SESSION 3 – NEW TOOLS & APPROACHES FOR MAIZE**<br>Chair: Karen McGinnis Talks 7-11. Pages 37-39 |
| 10:45 AM | **Johanna Smyth, Oregon State University** [T7]<br>*Proteomic profiling suggests control of translation and protein stability is crucial for pollen tube germination in maize (Zea mays)* |
| 11:05 AM | **Maike Stam, University of Amsterdam** [T8]<br>*Identification and characterization of distant enhancers in Zea mays* |
| 11:25 AM | **Sergei Svitashev, DuPont Pioneer** [T9]<br>*Maize genome editing using CRISPR/Cas9 technology* |
| 11:45 AM | **Elizabeth Lee, University of Guelph** [T10]<br>*Genomes to Fields' Maize GxE Project: Expression of productivity and phenological traits across a diverse set of environments* |
| 12:05 PM | **Addie Thompson, Purdue University** [T11]<br>*Beyond GWAS: Characterizing drought response of elite temperate and tropical maize using a systems-biology approach* |

## Friday, March 18 (continued)

12:30 PM – 1:30 PM **LUNCH** (Grand Ballroom 1-4)

1:30 PM – 5:00 PM **POSTER SESSION 1** (Ballroom Foyer and 3rd floor in St. Johns and Clearwater Rooms)

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

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

4:40 PM – 6:00 PM **SESSION 4 – GENOME STRUCTURE & CYTOGENETICS**
Chair: Ruairidh Sawers Talks 12-15. Pages 40-43.

4:40 PM **Zhikai Liang, University of Nebraska-Lincoln** [T12]
*B73 maize population structure analysis by RNA-seq data*

5:00 PM **Michelle Stitzer, University of California, Davis** [T13]
*Ecological determinants of transposable element distributions in the B73 maize genome*

5:20 PM **Gernot Presting, University of Hawaii** [T14]
*Resolving the centromere paradox – microevolution of centromeric DNA repeats*

5:40 PM **Arnaud Ronceret, Universidad Nacional Autónoma de México**[T15]
*Analysis of maize meiotic mutants to fix heterosis*

6:00 PM – 7:00 PM **DINNER** (Grand Ballroom 1-4)

7:00 PM – 9:00 PM **SESSION 5 – MCCLINTOCK PRIZE PRESENTATION**
Chair: Sherry Flint-Garcia McClintock Talk. Page 30

7:00 PM **Nathan Springer, MGEC Chair**
*Early- and Mid-Career Awards*

**Ron Phillips, University of Minnesota**
*McClintock Prize Presentation*

7:40 PM **Jeffrey D. Palmer, Indiana University at Bloomington** [M1]
*Horizontal gene transfer – the dark matter of plant genomes and plant evolution*

9:00 PM – 1:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY**
(Grand Ballroom 1-4)

## Saturday, March 19

7:00 AM – 8:00 AM **BREAKFAST** (Grand Ballroom 5-8)

8:00 AM – 10:20 AM **SESSION 6 – QUANTITATIVE GENETICS & BREEDING**
Chair: Jianbing Yan Talks 16-22. Pages 44-50.

8:00 AM **Jorge Alberto Romero Navarro, Cornell University** [T16]
*Identifying the diamond in the rough: Studying allelic variation for complex traits in maize landraces*

8:20 AM **Tingting Guo, Iowa State University** [T17]
*Optimal design of genomic prediction in maize hybrid breeding*

8:40 AM **Jason Wallace, The University of Georgia** [T18]
*The effect of host genetics on the maize leaf microbiome across 270 diverse inbred lines*

9:00 AM **Xiaohong Yang, China Agricultural University** [T19]
*Genome-wide association study reveals genetic basis and role of ZmVPP1 in drought tolerance in maize seedlings*

9:20 AM **Sebastien Praud, Biogemma** [T20]
*BALANCE, a powerful population to decipher complex traits in maize*

9:40 AM **Lei Liu, Huazhong Agricultural University** [T21]
*KRN4 controls quantitative variation in maize kernel row number*

10:00 AM **Shuhua Zhan, University of Guelph** [T22]
*The genetic basis and adaptive significance of transcript abundance differences between two maize inbreds*

10:20 AM – 10:45 AM **BREAK**

10:45 AM – 12:25 PM **SESSION 7 – CELL & DEVELOPMENTAL GENETICS**
Chair: Amanda Wright Talks 23-27. Pages 51-55.

10:45 AM **Karina van der Linde, Stanford University** [T23]
*Biomolecular interpretation of the Trojan horse myth: Use of Ustilago maydis to analyze the function of maize (Zea mays) MAC1*

11:05 AM **Pablo Martinez, University of California Riverside** [T24]
*TANGLED-1 function in maize*

11:25 AM **Kin Lau, Purdue University** [T25]
*Induced and natural variation in genes encoding the microtubule severing ATPase, katanin p60 (KTN1), alter meristem shape, plant morphology and spikelet density*

11:45 AM **Marisa Rosa, University of California at Berkeley** [T26]
*The maize Mid-Completing Activity protein NARROW ODD DWARF is required for normal plant growth and leaf pattering*

12:05 PM **Josh Strable, Iowa State University** [T27]
*Maize YABBY genes drooping leaf1 and drooping leaf2 affect agronomic traits by regulating leaf and floral architectures*

## Saturday, March 19 (continued)

12:30 PM – 1:30 PM **LUNCH** (Grand Ballroom 1-4)

1:30 PM – 5:00 PM **POSTER SESSION 2** (Ballroom Foyer and 3rd floor in St. Johns and Clearwater Rooms)

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

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

5:00 PM – 6:00 PM **COMMUNITY SESSION - Maize Genetics Executive Committee**
MGEC Chair: Nathan Springer (Grand Ballroom 5-8)

6:00 PM – 7:00 PM **DINNER** (Grand Ballroom 1-4)

7:00 PM – 8:55 PM **SESSION 8 – PLENARY TALKS**
Chair: Erich Grotewold Pages 28 & 29.

7:00 PM **ANNOUNCEMENTS** (Grand Ballroom 5-8)
David Braun

7:15 PM **Mark Stitt, Max Planck Institute for Molecular Plant Physiology, Golm, Germany** [Plen 3]
*Balancing the carbon budget: Are plants better than bankers and politicians?*

8:05 PM **Chris-Carolin Schön, Technische Universität München, Freising, Germany** [Plen 4]
*Splendor and misery of quantitative genetics in the era of high-throughput technologies*

9:00 PM – 2:00 AM **INFORMAL POSTER VIEWING/DANCE** (**Grand Ballroom 1-4**)

## Sunday, March 20

7:00 AM – 8:20 AM **BREAKFAST** (Grand Ballroom 1-4)

**Posters should be taken down by 9 am!**

8:20 AM – 9:50 AM **SESSION 9 – BIOCHEMICAL & MOLECULAR GENETICS I**
Chair: Petra Wolters Talks 28-31. Pages 56-59.

8:20 AM **ANNOUNCEMENTS** (Grand Ballroom 5-8)
David Braun

8:30 AM **Mihai Miclaus, National Institute of Research and Development for Biological Sciences, Romania** [T28]
*Maize cytolines provide key nuclear genes that are under the control of retrograde signaling pathways in plants*

8:50 AM **Janaki Mudunkothge, University of Florida** [T29]
*The maize dosage-effect defective kernel1 (ded1) locus encodes a MYB transcription factor controlling endosperm development and grain-fill*

9:10 AM **Charles Hunter, USDA-ARS** [T30]
*Maize white seedling 3 results from disruption of homogentisate solanesyl transferase*

9:30 AM **Richard Vierstra, Washington University in St. Louis** [T31]
*Autophagic recycling plays a central role in maize nutrient remobilization*

9:50 AM **BREAK**

10:20 AM – 11:30 AM **SESSION 10 – BIOCHEMICAL & MOLECULAR GENETICS II**
Chair: Wes Bruce Talks 32-34. Pages 60-62.

10:20 AM **Norman Best, Purdue University** [T32]
*Forward genetics identifies the nuclear pore complex component, aladin1, as necessary for tassel architecture and asymmetric cell division in maize*

10:40 AM **Hilde Nelissen, VIB Ghent University** [T33]
*Altered expression of the maize cytochrome P450 CYP78A1/KLUH increases biomass and seed yield by an extended duration of cell division*

11:00 AM **Jorge Nieto-Sotelo, Universidad Nacional Autónoma de México** [T34]
*Importance of mesocotyl and plumule growth on heat and drought avoidance in modern maize hybrids: physiology and GWAS*

11:30 AM **ADJOURNMENT**

# Posters

## Computational and Large-Scale Biology

| | | |
|---|---|---|
| *P1* | **Mary Schaeffer** <mary.schaeffer@ars.usda.gov> | *New Trait Data at MaizeGDB* |
| *P2* | **Carson Andorf** <carson.andorf@ars.usda.gov> | *MaizeGDB: New Tools and Resource* |
| *P3* | **Lisa Harper** <lisaharper@me.com> | *MaizeGDB Video Tutorials, Feedback Booth and Introducing the AgBioData Working Group* |
| *P4* | **Taner Sen** <taner.sen@ars.usda.gov> | *Breeder Survey, Tools, and Resources to Visualize Diversity and Pedigree Relationships at MaizeGDB* |
| *P5* | **Ethalinda Cannon** <ekcannon@iastate.edu> | *Stewardship of the Maize B73 Reference Genome Assembly* |
| *P6* | **Jack Gardiner** <jack.m.gardiner@gmail.com> | *Data Management and Analysis Solutions for Maize Predictive Phenomics: A partnership with the GxE Subgroup of the Genomes to Fields (G2F) Initiative* |
| *P7* | **Yinping Jiao** <yjiao@cshl.edu> | *A dramatically improved maize B73 reference genome constructed using single-molecule technologies* |
| *P8* | **Ning Yang** <yangningyingji@126.com> | *A maize and its wild relative (Zea.mays.Mexicana) genomes provide new insights for domestication, improvement and introgression* |
| *P9* | **Paul Chomet** <pchomet@nrgene.com> | *A new view of the maize genome that incorporates multiple denovo genome assemblies* |
| *P10* | **Hannah Worral** <hworral@iastate.edu> | *A storehouse of immeasurable worth: Breathing new life into archived GBS data* |
| *P11* | **Felix Francis** <felixfrancier@gmail.com> | *A thermo-align approach for the design of template-specific hybridization and priming oligonucleotides for repetitive genomes* |
| *P12* | **Jiaqiang Dong** <jqdong@waksman.rutgers.edu> | *Analysis of the zein gene family in maize W22 inbred line using single-molecule real-time sequencing technology* |
| *P13* | **Lin Li** <lixx1601@umn.edu> | *Ascertaining the Co-Expression Networks of Homeobox Genes in Maize Shoot Apical Meristem* |
| *P14* | **Christy Gault** <cg449@cornell.edu> | *Assembling the genome and transcriptome of maize's sister genus Tripsacum as a step toward identifying freezing tolerance genes* |
| *P15* | **Ni Jiang** <njiang@danforthcenter.org> | *Automatic imaging and 3D timeseries analysis of growing roots with shinyDR* |
| *P16* | **Kokulapalan Wimalanathan** <kokul@iastate.edu> | *Bulked Segregant - genotyping-by-sequencing: Cost-effective and background independent genetic mapping of mutants and QTL* |
| *P17* | **Pu Huang** <phuang@danforthcenter.org> | *$C_4$ gene discovery using cross species selection scan* |
| *P18* | **Robert Schaefer** <schae234@umn.edu> | *Camoco: systematic integration of co-expression and genome wide associations studies in Zea mays to detect causal variants.* |
| *P19* | **Christopher Lawrence** <cgl@iastate.edu> | *CGAT a CRISPR sgRNA Design Tool* |
| *P20* | **Daniel Carvalho** <danielsc@huskers.unl.edu> | *Comparative analysis of C4 photosynthesis genes in two independent origins of C4 in grasses* |

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| *P21* | **Yang Zhang**<br><yzhang91@unl.edu> | *Conservation and divergence of synthetic gene regulation in response to stress in maize and relatives* |
| *P22* | **Xianjun Lai**<br><xlai3@unl.edu> | *Conserved and lineage-specific alternative splicing of orthologous genes in maize, sorghum, and setaria* |
| *P23* | **A. Mark Settles**<br><settles@ufl.edu> | *Conserved gene targets between the maize RGH3 and human ZRSR2 RNA splicing factors* |
| *P24* | **Raeann Goering**<br><rgoering01@hamline.edu> | *Differential Expression of Maize Memory Genes and Phenotypic Change in Response to Priming and Cold Stress* |
| *P25* | **Keting Chen**<br><kchen@iastate.edu> | *Dissecting the metabolic network for silk extracellular cuticular lipids in maize: Contrasting linear regression models and correlation networks during silk development and different genotypes* |
| *P26* | **Emily Wear**<br><emily_wear@ncsu.edu> | *DNA replication dynamics in maize root tips* |
| *P27* | **Hank Bass**<br><bass@bio.fsu.edu> | *DNS - NUPRIME: Differential Nuclease Sensitivity for Nuclease Profiling as an Integrative Resource for Maize Epigenomics.* |
| *P28* | **Li Wang**<br><lilepisorus@gmail.com> | *Evolution of maize during post-domestication expansion across the Americas* |
| *P29* | **Kelly Swarts**<br><kls283@cornell.edu> | *Evolution of temperate maize in North America* |
| *P30* | **Avimanyou Vatsa**<br><akvhxd@mail.missouri.edu> | *Finding Groups of Phenotypes under Combined Stresses by Clustering* |
| *P31* | **Handong Su**<br><shdong@genetics.ac.cn> | *Fine scale nucleosome positioning coupled with centromeric chromatin in maize* |
| *P32* | **Alec Kollman**<br><akollman@iastate.edu> | *Gametophytic Cross Incompatibility in Maize: Sequencing the Ga1-m Locus* |
| *P33* | **Joseph Jeffers**<br><jeffe174@umn.edu> | *GeneWordSearch: An open source tool for keyword enrichment analysis of gene sets* |
| *P34* | **Jacob Washburn**<br><jdwr47@mail.missouri.edu> | *Genome-Guided Transcriptome Phylogenomics of the Paniceae Grasses* |
| *P35* | **Jingjuan Yu**<br><yujj@cau.edu.cn> | *Genome-wide analysis of the lysine biosynthesis pathway network during maize seed development* |
| *P36* | **Donald McCarty**<br><drm@ufl.edu> | *Genome-wide analysis of variation in gene-copy and paralog number in the HapMap2 collection of maize and teosinte inbreds* |
| *P37* | **Maria Mejia Guerra**<br><mejia-guerra.1@osu.edu> | *Genome-wide characterization of maize promoter regions and transposable element composition* |
| *P38* | **Md Shamimuzzaman**<br><mshamimuzzaman@danforthcenter.org> | *Genome-wide mapping of hypersensitive footprints in chromatin of developing maize tassels predicts regulators of inflorescence architecture* |
| *P39* | **Sandra Unterseer**<br><sandra.unterseer@tum.de> | *High-latitude adaptation through early floral induction in maize* |
| *P40* | **Qiuyue Chen**<br><qych@cau.edu.cn> | *Identification of genetic variants modulating alternative splicing in maize kernel* |
| *P41* | **Mingze He**<br><mhe@iastate.edu> | *Investigating diversity and possible functions of G-quadruplexes in regulatory regions of maize genes* |
| *P42* | **Shuai Zeng**<br><zengs@mail.missouri.edu> | *KBCommons: A multi 'OMICS' integrative framework for database and informatics tools* |

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|---|---|---|
| *P43* | **Kokulapalan Wimalanathan** <kokul@iastate.edu> | *Maize - GO Annotation Methods, Evaluation, and Review (Maize-GAMER)* |
| *P44* | **Qunfeng Dong** <qdong@luc.edu> | *MIGD: An Integrated Maize Inflorescence Genomics Database* |
| *P45* | **Jie Xu** <jiexu28@gmail.com> | *Natural antisense transcripts are significantly involved in regulation of drought stress in maize* |
| *P46* | **Fei Lu** <fl262@cornell.edu> | *Pan-genome analysis reveals functional variants in maize* |
| *P47* | **Jaclyn Noshay** <nosha003@umn.edu> | *Phylogenetic and transcriptome analysis of CBF and ICE gene families in maize* |
| *P48* | **Kevin Schneider** <kevinls@hawaii.edu> | *Polishing the highly repetitive centromere regions of Refgen V4* |
| *P49* | **Brian Smith-White** <smtwhite@ncbi.nlm.nih.gov> | *RefSeq Curation at NCBI - Adding Value to the Maize Genome Resources* |
| *P50* | **Alex Brohammer** <broha006@umn.edu> | *Relationship between genome and transcriptome variation and the predictive capacity of the pan transcriptome to the pan genome* |
| *P51* | **Thomas Brutnell** <tbrutnell@danforthcenter.org> | *Sequence, assembly and annotation of the maize W22 genome* |
| *P52* | **Nancy Manchanda** <nancym@iastate.edu> | *Sequencing, Assembly, and Annotation of Maize B104 : A Maize Transformation Resource* |
| *P53* | **Ningjing Tian** <ningjing@udel.edu> | *The free and open-source AGB platform for managing genetics and breeding research programs* |
| *P54* | **Greg Ziegler** <Greg.Ziegler@ars.usda.gov> | *Tools for leveraging GWAS dat afor knowledge discovery* |
| *P55* | **Xinxin Ding** <xding4@wisc.edu> | *Transcriptome profiling and regulatory network analysis of maize aleurone and starchy endosperm cells at different developmental stages* |
| *P56* | **Sivanandan Chudalayandi** <csiva@iastate.edu> | *Transcriptomic Analyses of Maize-Southern Corn Leaf Blight Interaction* |
| *P57* | **Indrajit Kumar** <ikumar@danforthcenter.org> | *Translational dynamics of nuclear genes during leaf development in maize* |
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*P108* **Bryan Gibbon** <bryan.gibbon@famu.edu>
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*P109* **Shan Jin** <szj133@psu.edu>
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*P110* **Rentao Song** <rentaosong@staff.shu.edu.cn>
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*P111* **Fan Feng** <fengfan_102@163.com>
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*P112* **Shawn Christensen** <shawn.christensen@ars.usda.gov>
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*P113* **Nathaniel Boyer** <nrb2bd@mail.missouri.edu>
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*P117* **Umnia Mahgoub** <umahgoub@iastate.edu>
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*P118* **Christine Shyu** <CShyu@danforthcenter.org>
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*P120* **Brian Dilkes** <bdilkes@purdue.edu>
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*P121* **Jutta Baldauf** <Baldauf@uni-bonn.de>
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*P122* **Yongrui Wu** <yrwu@sibs.ac.cn>
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*P123* **Fabian Strauss** <frs6493@louisiana.edu>
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*P125* **Eliecer Gonzalez** <egonzalez@langebio.cinvestav.mx>
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*P126* **Miaomiao Fu** <mmfu@sibs.ac.cn>
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*P127* **Brianna Griffin** <bdg13@my.fsu.edu>
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*P128* **Erin Mattoon** <em636@cornell.edu>
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*P130* **Aixia Li** <ali3@unl.edu>
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*P131* **Jiechen Wang** <jcwang@sibs.ac.cn>
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*P132* **Bri Vidrine** <bvidrine@iastate.edu>
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*P133* **Abiskar Gyawali** <abiskar.gyawali@sdstate.edu>
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*P134* **Qiujie Liu** <liuqiujie08@gmail.com>
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*P135* **Cairo Archer** <cma235@cornell.edu>
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*P136* **Junya Zhang** <zhangjunya@ufl.edu>
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*P137* **Christian Brigolin** <cjbrigolin@oakland.edu>
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*P138* **Colton McNinch** <cmcninch@iastate.edu>
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*P139* **John Gray** <jgray5@utnet.utoledo.edu>
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*P140* **Kevin Chu** <chu16@purdue.edu>
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*P141* **Ying Xie** <xieying890327@yahoo.com>
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*P144* **Quan Zhang** <qzhang@danforthcenter.org>
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*P145* **Daniel Frailey** <dfrailey@uga.edu>
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*P146* **Roselyn Hatch** <hatchr@purdue.edu>
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*P147* **Edward Ross** <ehross3@illinois.edu>
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*P148* **Kristen Leach** <leachka@missouri.edu>
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*P149* **Xiaoying Pan** <panxiaoying0707@126.com>
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*P150* **Weiwei Qi** <weiweiqi@shu.edu.cn>
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*P152* **Nelson Garcia** <ngarcia@waksman.rutgers.edu>
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*P153* **Hongyan Zheng** <pinkwinter@163.com>
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*P154* **John Hodge** <jghodge@okstate.edu>
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*P155* **Aurelie Grimault** <agrimault@carnegiescience.edu>
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*P156* **Alexander Gregory** <apggh5@mail.missouri.edu>
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*P157* **Paula McSteen** <mcsteenp@missouri.edu>
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*P158* **Erin Sparks** <erin.sparks@duke.edu>
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*P159* **Amanda Blythe** <amb4x2@mail.missouri.edu>
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*P160* **Carolyn Rasmussen** <carolyn.rasmussen@ucr.edu>
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*P161* **China Lunde** <lundec@berkeley.edu>
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*P162* **Mei Zhang** <mzhang11@stanford.edu>
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*P163* **Pengfei Qiao** <pq26@cornell.edu>
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*P164* **Yingying Cao** <ycao@danforthcenter.org>
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*P165* **Michael Muszynski** <mgmuszyn@hawaii.edu>
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*P166* **Fang Xu** <fxu@cshl.edu>
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*P167* **Carolyn Rasmussen** <carolyn.rasmussen@ucr.edu>
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*P168* **Beth Thompson** <thompsonb@ecu.edu>
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*P169* **Carla Coelho** <ccoelho@danforthcenter.org>
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*P171* **Chunhui Xu** <chunhuixu@sdu.edu.cn>
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*P176* **Aaron Sluis** <amsluis@gmail.com> — *Hoja loca is an AUX/IAA mutant that affects lateral organ initiation*

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*P187* **Dale Brunelle** <dale.brunelle@und.edu> — *Maize embryo morphogenesis: a mutational analysis*

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*P196* **Jacob Brunkard** <jake.brunkard@gmail.com>
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*P197* **Feng Sun** <epusun@sdu.edu.cn>
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*P198* **Ramesh Katam** <ramesh.katam@famu.edu>
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*P200* **Johanna Smyth** <smythj@science.oregonstate.edu>
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*P201* **Anding Luo** <aluo@uwyo.edu>
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*P202* **Chao Feng** <fengchao@genetics.ac.cn>
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*P203* **Michael Lewis** <mluudensis@gmail.com>
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*P204* **Davide Sosso** <dsosso@carnegiescience.edu>
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*P205* **JIngjuan Yu** <yujj@cau.edu.cn>
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*P206* **Chuanmei Zhu** <czhu@danforthcenter.org>
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*P207* **Alyssa Anderson** <alyssa.amy@berkeley.edu>
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*P208* **Madelaine Bartlett** <mbartlett@bio.umass.edu>
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*P209* **Dennis Zhu** <dxzc65@mail.missouri.edu>
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*P210* **Zhaobin Dong** <dongz@berkeley.edu>
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*P211* **Zong-Liang Chen** <zlchen@sdu.edu.cn>
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*P212* **Dongfang Wang** <dwang@spelman.edu>
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*P213* **Sarah Anderson** <snan@ucdavis.edu>
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*P214* **Dave Stateczny** <dave.stateczny@uni-hamburg.de>
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*P215* **Eden Johnson** <ejcv4@mail.missouri.edu>
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*P216* **Gregg Hoffman** <hoffman@psy.fsu.edu>
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*P217* **Andrea Gallavotti**
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*P218* **Muriel Longstaff**
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*P219* **Oliver Bear Don't Walk IV**
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*P220* **Antony Chettoor**
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*P221* **Katy Guthrie**
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*P222* **Qingyu Wu**
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*P224* **Elizabeth Hood**
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*P225* **Hannes Claeys**
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*Using natural variation and forward genetics to extend genetic networks controlling maize inflorescence development*

*P226* **Michelle Facette**
<mfacette@ucsd.edu>
*Using proteomics, network analysis and an enhancer screen to identify new mutants promoting maize subsidiary cell polarity*

*P227* **Peter Rogowsky**
<peter.rogowsky@ens-lyon.fr>
*Virtual 3D histology of developing maize seeds using X-ray in-line phase tomography*

*P228* **Angus Vajk**
<vajking@berkeley.edu>
*What is fun?*

*P229* **Jingjuan Yu**
<yujj@cau.edu.cn>
*ZmESDP, a maize endosperm-specific Dof protein gene, regulates aleurone development and starch accumulation in maize endosperm*

*P230* **Edgar Demesa-Arevalo**
<edemesaa@cshl.edu>
*ZmSCAFFOLD ATTACHMENT FACTOR B and ZmSCAFFOLD ATTACHMENT FACTOR B-LIKE and their putative role in maize inflorescence meristem development*

## Cytogenetics

*P231* **Jing Zhang**
<zhangjing@genetics.ac.cn>
*A cohesin subunit may facilitate centromere pairing in early meiotic prophase in maize*

*P232* **Natalie Nannas**
<njnannas@uga.edu>
*Asymmetric anaphase segregation of chromosomes in male maize meiosis*

*P233* **Yalin Liu**
<ylliu@genetics.ac.cn>
*Centromere misdivision, chromosome breakage and pollen irradiation promote de novo centromere formation in maize*

*P234* **Adam Johnson**
<afj8c8@mail.missouri.edu>
*Chromosome dosage effects on the transcriptome*

*P235* **Jonathan Gent**
<gent@uga.edu>
*Distinct kinesins on Abnormal Chromosome 10 are candidates for driving neocentromere activity of knob180 and TR-1 knobs*

*P236* **Hefei Wang**
<hfwang@genetics.ac.cn>
*Dynamic changes of Structural Maintenance of Chromosomes, smc2 and smc4, during the cell cycle of maize*

*P237* **Morgan McCaw**
<mem7b6@mail.missouri.edu>
*Fast-Flowering Mini-Maize: Seed to Seed in 60 Days Update*

*P238* **Mischa Olson**
<mao83@cornell.edu>
*Meiotic recombination in synthetic tetraploid lines of maize*

*P239* **Seth Applegate**
<sethap@kwc.edu>
*Mutations within Pseudogenes of Various Ab10 Haplotypes*

*P240* **Yang Liu** <yangliu@genetics.ac.cn>
*Phosphorylation of histone H3 Thr3 by Haspin is correlated with cohesion during the cell cycle*

*P241* **Katherine Easterling** <kae09@my.fsu.edu>
*W22 Pachytene FISH Karyotype; Meiotic Chromosomes & Somatic Chromatin.*

## Education & Outreach

*P242* **Jolie Wax** <jwax@udel.edu>
*Framework for a Genome Science Major, Referencing Maize Research for Education*

*P243* **Peter Rogowsky** <peter.rogowsky@ens-lyon.fr>
*Genome ENgineering Improvement for Useful plants of a Sustainable agriculture*

*P244* **Amado Estrada** <ale12@my.fsu.edu>
*Genome strolling through the Maize-10-Maze; a living museum outreach project exhibiting select mutants of maize*

*P245* **Thomas Slewinski** <thomas.l.slewinski@monsanto.com>
*Monsanto Academic Outreach*

*P246* **Carolyn Lawrence-Dill** <triffid@iastate.edu>
*P3: Predictive Phenomics in Plants, A NSF Research Traineeship Program*

*P247* **Denise Costich** <d.costich@cgiar.org>
*The International Maize Genetic Resources Advisory Committee (IMGRAC) Meeting at CIMMYT: Implementing a global strategy for the maize community*

## Quantitative Genetics & Breeding

*P248* **Darlene Sanchez** <darlenes@iastate.edu>
*A comparison between GBS and SNP chip marker systems in molecular profiling of doubled haploid exotic introgression lines in maize*

*P249* **Diego Jarquin** <jhernandezjarquin2@unl.edu>
*A multi-institution multi-year collaboration to study the genotype-by-environment interaction in maize across a diverse set of hybrids, locations and years.*

*P250* **Ying Ren** <renying900115@huskers.unl.edu>
*Accelerated development of Quality Protein Popcorn (QPP)*

*P251* **Ruth Wagner** <ruth.wagner@monsanto.com>
*Accelerating Commercial Plant Breeding and Product Development Through Genomics Data*

*P252* **Jiahn-Chou Guan** <guanjc@ufl.edu>
*An Unexplored Avenue of Striga-Resistance in Maize*

*P253* **Santiago Alvarez Prado** <santiago.alvarez-prado@supagro.inra.fr>
*Assessing genetic variability for maize growth related traits and sensitivity to water deficit in phenotyping platform.*

*P254* **Junping Chen** <junping.chen@ars.usda.gov>
*Association analysis of field heat tolerant traits in maize and sorghum*

*P255* **Addie Thompson** <thomp464@purdue.edu>
*Beyond GWAS: Characterizing Drought Response of Elite Temperate and Tropical Maize using a Systems-Biology Approach*

*P256* **Chutinan Jaroenchai** <cjaroenc@uoguelph.ca>
*Biomass Dynamics of Long-Ear Genetics*

*P257* **Songlin Hu** <hsonglin@iastate.edu>
*Brassinosteroid and Gibberellin control of plant height in a phenotypic-selected introgression library in maize*

*P258* **Laura Morales** <lm596@cornell.edu>
*Brown midrib2: a role in resistance to Fusarium ear rot and fumonisin contamination*

*P259* **Manfred Mayer** <manfred.mayer@tum.de>
*Characterization of genetic diversity, population structure and linkage disequilibrium within and across 35 European maize landraces using high-density genomic data*

*P260* **Joseph Knoll** <Joe.Knoll@ars.usda.gov>
*Combining Ability of New Maize Lines for Yield and Aflatoxin Resistance*

*P261* **Julia Winkeler** <jwink@udel.edu>
*Conditioning Tests for Selection with Information on Segregation Distortion*

*P262* **Qing Li** <qing.li@doane.edu>
*Detecting changes in maize root exudate composition after cold stress using NMR and Principal Components Analysis*

*P263* **Jianbing Yan** <yjianbing@mail.hzau.edu.cn>
*Development of a maize MAGIC population and applications in quantitative traits dissection*

*P264* **Mark Holmes** <holme616@umn.edu>
*Development of a phenotypic platform to evaluate kernel processing quality for corn chip production*

*P265* **Rajanikanth Govindarajulu** <ragovindarajulu@mail.wvu.edu>
*Dissecting the genetic underpinnings of tillering in a sorghum RIL population [S. bicolor (Tx7000) × S. propinquum]: a preliminary report*

*P266* **Aaron Kusmec** <amkusmec@iastate.edu>
*Divergent Selection for Shoot Apical Meristem (SAM) Size*

*P267* **Jennifer Arp** <jarp2@illinois.edu>
*Dynamic regulatory changes in nitrogen utilization genes from a century of selection for seed protein concentration in maize*

*P268* **Jorge Torres** <jtorres@langebio.cinvestav.mx>
*Effect of teosinte parviglumis introgression into a B73 background on root system architecture under low phosphorus conditions*

*P269* **Sanzhen Liu** <liu3zhen@ksu.edu>
*Elucidation of the Genetic Landscape of Goss's Bacterial Wilt Resistance Via Genome-wide Association as well as Genome Sequencing of Extremely Phenotypic Bulks*

*P270* **Avinash Karn** <akarn@mail.missouri.edu>
*Evaluating Teosinte Alleles for Kernel Composition in Maize*

*P271* **Jessica Bubert** <jbubert2@illinois.edu>
*Evaluating the impact of heterozygosity on nitrogen use efficiency traits in maize*

*P272* **Sailaja Maddali** <saili71@uga.edu>
*Evaluation of Setaria viridis germplasm for herbicide resistance*

*P273* **Mona Mazaheri** <mmazaheri@wisc.edu>
*Expanding the Wisconsin diversity panel to improve GWAS in maize*

*P274* **Guoying Wang** <wangguoying@caas.cn>
*Exploring the genetic basis of maize drought tolerance by genome-wide association study and linkage mapping*

*P275* **Adam Bray** <abray@danforthcenter.org>
*Exposing the hidden half: What can we learn from high-throughput root imaging techniques?*

*P276* **Karl Kremling** <kak268@cornell.edu>
*Expression in 7 tissues from 300 lines reveals functional regulatory variants (eQTL)*

*P277* **Flor Acevedo** <floredith.acevedo@gmail.com>
*Fall armyworm herbivory affects silica accumulation in corn and rice*

*P278* **Seth Murray** <sethmurray@tamu.edu>
*Field based phenotyping using unmanned aerial vehicles (UAVs) and ground vehicles, what are we measuring and what have we learned?*

*P279* **Jeffery Gustin** <jgustin@ufl.edu>
*Finding the Haploid Needle in a Hybrid Haystack: Discrimination of Haploid Maize Kernels by Single Kernel Near-Infrared Spectroscopy*

*P280* **Alessandra York** <torno@wisc.edu>
*Fine-mapping a major maize domestication QTL for ear diameter*

*P281* **Yongrui Wu** <yrwu@sibs.ac.cn>
*Gene duplication at the 27-kDa γ-zein locus is associated with artificial selection for quality protein maize*

*P282* **Nina Chumak** <nina.chumak@botinst.uzh.ch>
*Generating clonal progeny in maize*

*P283* **Anna Glowinski** <acs5fd@mail.missouri.edu>
*Genetic analysis of doubled haploids derived from the Zea Synthetic population*

*P284* **Jiaojiao Ren** <renjiaojiao789@sina.com>
*Genetic Analysis of Haploid Fertility in Maize*

*P285* **Mercy Kabahuma** <kabahuma@iastate.edu>
*Genetic analysis of quantitative disease resistance in maize against two isolates of northern corn leaf blight (Setosphaeria turcica)*

*P286* **Meng Yujie** <mengyujie25@126.com>
*Genetic architecture exploration of rind penetrometer resistance and in vitro dry matter digestion in maize*

*P287* **Michael Tuholski** <tuholski.mike@gmail.com>
*Genetic Architecture of the Maize Inflorescence Shattering Trait*

*P288* **Alain Charcosset** <charcos@moulon.inra.fr>
*Genetics of hybrid performance in maize: QTL detection for biomass production in a reciprocal multiparental design*

*P289* **Matheus Baseggio** <mb2446@cornell.edu>
*Genome-wide association study of carotenoid and tocochromanol levels in sweet corn kernels*

*P290* **Yingjie Xiao** <shanren0179@163.com>
*Genome-wide dissection of the maize ear genetic architecture using multiple populations*

*P291* **Zhengbin Liu** <zliu@danforthcenter.org>
*Genome-wide Study of Genes Controlling Phenotypic Plasticity of Root Architecture to Nitrogen in Maize*

*P292* **Lorena Rios-Acosta** <lrios@illinois.edu>
*Genotypic diversity in yield and grain quality responses to elevated ozone of diverse inbred and hybrid maize*

*P293* **Shumeng Zhang** <sz88391@uga.edu>
*GMATA: a powerful tool for microsatellite characterization, marker development and graphic display applied to the maize genome*

*P294* **Thomas Lubberstedt** <thomasl@iastate.edu>
*Haploid Inducer Development for Specialty Corn*

*P295* **Joseph Gage** <jgage2@wisc.edu>
*High-throughput Image-based Phenotypic Analysis of Tassel Morphology in Maize*

*P296* **Matthew Dzievit** <mdzievit@iastate.edu>
*High-throughput phenotyping and genotyping to dissect canopy architecture in maize*

*P297* **Nancy Wahl** <nancyjwahl7@gmail.com>
*Identification of Aflatoxin Resistance and High Yield Potential in Maize Hybrids in the Southeast Regional Aflatoxin Trials (SERAT)*

*P298* **Peter Balint-Kurti** <pjbalint@ncsu.edu>
*Identification of genes associated with quantitative and multiple disease resistance in maize*

*P299* **Calli Anibas** <canibas@wisc.edu>
*Identification of Genetic Background Modifiers of the Maize brown midrib3 (bm3) Mutant*

*P300* **Carrie Butts-Wilmsmeyer** <cjbutts2@illinois.edu>
*Improving the nutritional quality of processed maize food products: A step toward the prevention of aging-related diseases in humans*

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|---|---|
| *P301* **Alison Cooke** <acooke01@uoguelph.ca> | *In silico mapping of yield quantitative trait loci (QTL) in maize* |
| *P302* **Fabian Strauss** <frs6493@louisiana.edu> | *In vitro regeneration of Sorghum plants from immature embryo – A potential tool for sorghum transformation* |
| *P303* **Jinliang Yang** <jolyang@ucdavis.edu> | *Incorporation of Evolutionary Constraint Improves Genomic Prediction of Hybrid Phenotypes* |
| *P304* **Danilo Hottis Lyra** <daniloh@iastate.edu> | *Integrating traits associated to nitrogen use efficiency into the genomic prediction of tropical maize lines* |
| *P305* **Scott Stelpflug** <scott.c.stelpflug@monsanto.com> | *Integrating 'omics' data to reveal genotype-phenotype associations underlying maize seed imbibitions traits* |
| *P306* **Heather Manching** <hcorn@udel.edu> | *Investigating the genetic basis of parallel response to selection for early flowering time in the TropicS.* |
| *P307* **Claude Welcker** <claude.welcker@supagro.inra.fr> | *Investigating the unstability of yield-related QTL in maize: toward identification of genomic regions associated with tolerance to precise scenarios of heat or drought* |
| *P308* **Addie Thompson** <thomp464@purdue.edu> | *Maize germplasm organization and visualization according to genomewide marker effects* |
| *P309* **Anna Krzywdzinski** <akrzywdz@uoguelph.ca> | *Mapping and identifying candidate genes of the modifier of amylose extender 1 (MAE1) mutation in maize (Zea mays L.)* |
| *P310* **Tyr Wiesner-Hanks** <tw372@cornell.edu> | *Mapping of quantitative disease resistance loci derived from recurrent selection for northern leaf blight resistance* |
| *P311* **Michael Anokye** <anomic17@yahoo.com> | *Mapping variation linked to phosphate efficiency among maize landraces originating from the Purhepecha plateau of Michoacan* |
| *P312* **Terra Hartman** <terra.hartman@doane.edu> | *Measuring the growth and development of Maize inbreds B73 and Mo17 in a newly established outdoor corn plot after cold stress delivered in early seedling development* |
| *P313* **Martin Garcia-Flores** <masterfoodscience@live.com> | *Metabolic Profiling of Plant Extracts Using Direct Injection Electrospray Ionization Mass Spectrometry Allows for High-Throughput Phenotypic Characterization According to Genetic and Environmental Effects* |
| *P314* **Tes Posekany** <posekany@iastate.edu> | *Metabolite-QTL analysis of cuticular surface lipid production on maize silks* |
| *P315* **Ginnie Morrison** <morrisong@missouri.edu> | *Mild Inbreeding Depression in the Zea Synthetic Population* |
| *P316* **Abebe Menkir** <a.menkir@cgiar.org> | *Mining allies in exotic germplasm to modify carotenoid profile and content in tropical maize* |
| *P317* **Xuecai Zhang** <xc.zhang@cgiar.org> | *Molecular characterization of CIMMYT maize inbred lines with genotyping-by-sequencing SNPs* |
| *P318* **Li Wei** <welliongo@qq.com> | *Morphological observation of twin embryos generated by in vivo haploid induction in maize* |
| *P319* **Xiaoqing Yu** <xyu@iastate.edu> | *Multivariate Prediction and Optimization of Validation Population in Genomic Selection* |
| *P320* **Alison Cooke** <acooke01@uoguelph.ca> | *Parental analysis of sibling genomes from a maize breeding program* |
| *P321* **Jonathan Renk** <jrenk@wisc.edu> | *Phenotypic variation and genetic dissection of silage yield and compositional traits in recombinant inbred testcrosses in maize. (Zea mays L.)* |
| *P322* **Luis Lopez_Zuniga** <lolopezz@ncsu.edu> | *Production of Chromosome Segment Substitution Lines for the identification of multiple disease resistance loci in Maize* |

*P323* **Alexandra Asaro** <aasaro@wustl.edu>
*QTL by Environment Interactions Underlying the Kernel Ionome in Maize*

*P324* **Rafael Espejel-Venado** <oespejel@purdue>
*QTL Mapping for levels of β-cryptoxanthin in a Biparental Population*

*P325* **Andrew Doust** <andrew.doust@okstate.edu>
*QTL mapping of branching traits in Setaria populations grown in multiple environments, and comparison of QTL to those found in switchgrass, sorghum and maize*

*P326* **Lauren Stutts** <laurenrstutts@ufl.edu>
*Quantitative genetic analysis of the NC350 x B73 recombinant inbred line (RIL) population using single-kernel near infrared spectroscopy (NIR)*

*P327* **Ramesh Dhakal** <rdhakal06@gmail.com>
*Quantitative trait loci (QTL) for reducing aflatoxin accumulation in corn*

*P328* **Chin Jian Yang** <cyang227@wisc.edu>
*Quantitative Trait Locus (QTL) Mapping of Domestication Traits in Multiple Maize-Teosinte $BC_1S_4$ Populations*

*P329* **Vivek Shrestha** <vivek.shrestha@sdstate.edu>
*Quantitative Trait Polymorphisms Emerging from Doubled Haploids Maize Lines.*

*P330* **Christian Escoto** <christian.escoto@ira.cinvestav.mx>
*Root Architecture of maize (Zea mays) in conditions in different concentrations of phosphorus.*

*P331* **Ana L Alonso Nieves** <ana.alonso@langebio.cinvestav.mx>
*Root morphology and phosphate homeostasis in maize: the role of the ZmMed12a and ZmMed12b genes*

*P332* **Matthew Murray** <mmurray7@wisc.edu>
*Signatures of selection in sweet maize suggest a single donor shapes modern elite se1 inbreds*

*P333* **Yibing Yuan** <yibingyuan.yuan@gmail.com>
*Simple and quick method for GBS genotype imputation in bi-parental populations for linkage map construction in maize*

*P334* **Gladys Cassab** <gladys@ibt.unam.mx>
*Synergy between root hydrotropic response and root biomass enhances grain yield in maize (Zea mays L.)*

*P335* **Max Feldman** <mfeldman@danforthcenter.org>
*Temporal Monitoring in Controlled and Field Environments Reveals Dynamic Genetic Factors Underlying Plant Height in the C4 Model Grass Setaria*

*P336* **Natalia de Leon** <ndeleongatti@wisc.edu>
*The Effect of Artificial Selection on Phenotypic Plasticity: The Genotype by Environment Interaction Project in Maize*

*P337* **Brett Burdo** <burdo@wisc.edu>
*The Effect of Crossing Strategy on Genomic Prediction in Maize*

*P338* **Dan Li** <qdlidan@126.com>
*The genetic architecture of leaf number and its genetic relationship to flowering time in maize*

*P339* **Ann Stapleton** <stapletona@uncw.edu>
*The genetics of just right: dose-response curve fits to drought and nitrogen limitation applied together allow mapping of loci that exhibit synergistic and antagonistic responses to stress combinations*

*P340* **Peter Balint-Kurti** <pjbalint@ncsu.edu>
*The Genetics of leaf flecking in maize and its relationship to the defense response and broad-spectrum disease resistance*

*P341* **Carolina Cintora** <cimca1218@hotmail.com>
*The role of teosinte (Zea mays spp. mexicana) introgression as a driver of stem pubescence in Mexican highland maize*

*P342* **Vivek Shrestha** <vivek.shrestha@sdstate.edu>
*The search for modifiers of the maize gametophyte factor (Ga1-S).*

*P343* **Alina Ott** <aott@iastate.edu>
*Three-Fourths of Maize Presence-Absence Variants (PAVs) are not in Linkage Disequilibrium (LD) with Nearby SNPs*

*P344* **Silvio Salvi** <silvio.salvi@unibo.it>
*Toward cloning a major QTL for flowering time on maize chromosome 3*

*P345* **Yuanyuan Chen** <yychen@tamu.edu>
*Validating SNPs controlling maize grain yield and plant height in Texas hybrid testcrosses*

*P346* **Cinta Romay** <mcr72@cornell.edu>
*What controls yield in NAM and Ames maize hybrids?*

*P347* **Nathanael Ellis** <nellis@danforthcenter.org>
*Whole plant phenotyping of maize diversity lines in controlled water stressed environments*

## Transposons & Epigenetics

*P348* **Mithu Chatterjee** <cmithu@waksman.rutgers.edu>
*A sequenced-indexed reverse genetics resource for maize*

*P349* **Linda Stroud** <lstroud@bio.fsu.edu>
*Altered DNA accessibility in B73, W22 and mutant genotypes*

*P350* **Weijia Su** <weijia@iastate.edu>
*Analysis of the Composite Insertion —A Novel Structure Generated by Alternative Transposition*

*P351* **Cory Hirsch** <cdhirsch@umn.edu>
*Comprehensive characterization of the relationship between transposable elements and genic regions in maize*

*P352* **Daymond Parrilla** <parrilld@usca.edu>
*Determining the Sequences That Induce Hyperactive Transposition of mPing*

*P353* **Stephanie Diaz** <diazss@usca.edu>
*Developing mPing-based activation tags*

*P354* **Stefania Vendramin Alegre** <svendramin@bio.fsu.edu>
*Epigenetic regulation mediates transcriptional responses to abscisic acid in Zea mays*

*P355* **Dafang Wang** <wang2630@purdue.edu>
*Exploring the black box of siRNA communication between maize endosperm and embryo using B-A translocation lines.*

*P356* **Cristian Forestan** <cristian.forestan@unipd.it>
*Exploring the stress-induced, epigenetic-regulated genome stability and epigenomic variability in maize*

*P357* **Meixia Zhao** <zhao185@purdue.edu>
*Genome wide analysis of small RNAs reveals dynamic changes during vegetative phase change in maize*

*P358* **Lauren Schulte** <lschulte@bio.fsu.edu>
*Genome-wide analysis of RNA Polymerase II occupancy in Zea mays*

*P359* **Jason Lynn** <jlynn@bio.fsu.edu>
*Genome-wide analysis of RNA polymerase-IV:Mop1 binding in Zea mays*

*P360* **Jay Hollick** <hollick.3@osu.edu>
*GROseq and RNAseq comparisons identify RNA stability differences among seedling-expressed genes*

*P361* **David Gilbert** <gilberdm@usca.edu>
*Investigating the transposition mechanism of the MITE mPing*

*P362* **Mary Daliberti** <m.daliberti@ufl.edu>
*Mapping and genetic transmission of maternal rough endosperm (mre) mutants that display parent-of-origin effects in maize kernel development*

*P363* **Wenwei Xiong** <xiongwe@mail.montclair.edu>
*Massive rolling-circle amplification of Helitrons in plant genomes*

*P364* **Dhanushya Ramachandran** <dramacha@mix.wvu.edu>
*Methods for accurate quantification of LTR-Retrotransposons copy number using short-read sequence data: a case study in Sorghum*

*P365* **Jonathan Saunders** <jonosaun@ufl.edu>
*Over 8,000 new maize mutants in Release #8 from Uniform Mu*

*P366* **Fang Bai** <fbai001@ufl.edu>
*Parent-of-origin effect mutants regulating endosperm cellular development in the maize seed*

*P367* **Natalie Deans**
<deans.11@osu.edu>
*Proper ontogeny and maintenance of paramutant states in Zea mays is defined by required to maintain repression3 function*

*P368* **Minkyu Park**
<minkju@hanmail.net>
*Sample sequence analysis of grass genomes indicates frequent and repeated horizontal transfer of LTR-retrotransposons*

*P369* **Bosen Zhang**
<bszhang@illinois.edu>
*Small RNA sequencing reveals novel regulatory diversity in maize*

*P370* **Elizabeth Buescher**
<ebuesche@purdue.edu>
*sRNA from the female gametophyte impacts the epigenome at fertilization affecting seed survival*

*P371* **Kameron Wittmeyer**
<ktw5072@psu.edu>
*The mysterious Ufo1: What we have learned from global analyses*

*P372* **Victor Raboy**
<victor.raboy@ars.usda.gov>
*Trans-Generational Inheritance in Response to Macronutrient Stress in Barley and Maize*

*P373* **Jin Cui**
<juc326@psu.edu>
*Transcriptome Analysis of Ufo1 Identify Epigenetically Impacted Genes*

*P374* **Domitille Chalopin**
<chalopin@uga.edu>
*Transposable element evolution in the bambusoideae*

*P375* **Nicholas Heller**
<njhelle2@illinois.edu>
*Variation and Heritability in a Population of Epigenetic NILs*

## Late Posters

*P376* **Asha Cotterell**
<acottere@scmail.spelman.edu>
*Promoter deletion analysis of an invertase inhibitor during Arabidopsis seed development*

# Plenary Talk Abstracts

**Plenary 1** **Thursday, March 17 7:15PM**

## Sugar in your ears: Regulating transport, metabolism, and consequences

(presenter: Karen E. Koch <kekoch@ufl.edu>)
Full Author List: Koch, Karen E.[1]
[1] Plant Molecular and Cellular Biology, Horticultural Sciences, UF Genetics Institute, University of Florida, Fifield Hall, 2550 Hull Rd., Gainesville, FL, 32611

Maize yield depends on sucrose movement into kernels. We are rapidly gaining new insights into this movement, its regulation, and its broad network of consequences. Key advances center on the following: 1) The original Shannon hypothesis for sucrose cleavage at the kernel base has expanded to include developmental changes, sugar cycling, and metabolite signaling. 2) Sugar-responsive genes are now known to modulate processes ranging from export functions of source leaves (eg. tie-dyed mutants) to import capacity of kernels. The latter include developmental effects of sugar signals operating at the transcriptional level and via other mechanisms well beyond substrate alone. 3) Identification of an extremely low-oxygen state throughout the endosperm indicates that the starch and protein deposition in this structure may proceed in a very different metabolic environment than previously envisioned. 4) Maternal tissues can affect sucrose delivery to kernels by a range of mechanisms, one being strigolactone control of structural features. This hormone reduces physical constraints to kernel expansion, appears to alter physical capacity for flux through the path for sucrose delivery, and may have been under selection during domestication. 5) Genes for diverse sucrose and hexose transporters have been identified in the sugar-transfer zones of maize kernels, with at least one of these (SWEET4c) showing a domestication signature and a critical role in kernel growth. Together, these advances deepen our appreciation of multiple mechanisms that put sugar in our ears.

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## Roots of the Second Green Revolution

(presenter: Jonathan P. Lynch <jpl4@psu.edu>)
Full Author List: Lynch, Jonathan P.[1]
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The historical Green Revolution was an input intensification strategy combining fertilizers with fertilizer responsive crop genotypes, whereas the second Green Revolution will improve crop productivity with limited nutrient and water inputs. Stress tolerant, resource-efficient crops are urgently needed in global agriculture. Recent advances in root biology open the possibility of breeding crop varieties with root phenotypes improving the acquisition of key resources (water, N, P) and therefore greater yield in stressful environments. Root phenes improving topsoil foraging substantially improve P capture and are being deployed in legume breeding programs in the tropics. Root phenes improving rapid subsoil exploration can substantially improve water and N capture, and are beginning to be deployed in maize breeding programs in Africa. An emerging paradigm is that the minimization of root metabolic costs is an effective path to root phenotypes with superior soil exploration and resource capture. High throughput phenotyping platforms have been developed which are identifying major genes controlling these phenotypes. Although the fitness landscape of root phenotypes is complex, the development of new crop varieties with root phenotypes improving soil resource capture is having impact now and promises to be an important element of the resilient, sustainable cropping systems needed in the 21st century.

## Balancing carbon Budgets: Are Plants better than our Politicians and Bankers?

(presenter: Mark Stitt <mstitt@mpimp-golm.mpg.de>)
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Plants adjust their metabolism and growth to a fluctuating environment. I will focus on how they adjust to a changing supply of carbon. I will start by considering how carbon supply and growth are coordinated during vegetative growth. During the day, plants carry out photosynthesis. Part of the fixed carbon is temporarily stored as starch, and remobilized to support metabolism and growth at night. It has been known since the 1980's that starch breakdown is regulated such it is almost but not totally exhausted at the end of the night. This optimizes growth while minimizing the risk of periods of carbon starvation. I will discuss how we have used a range of 'omics approaches to characterize this response and have identified a key role for the biological clock in regulating the rate of starch breakdown and the timing of growth during diurnal cycles. While doing this I will introduce new methods for measuring expansion growth, and for quantifying the rates of protein and cell wall synthesis. I will then discuss the role of the sugar-signaling molecule trehalose-6-phosphate in adjusting metabolism and allocation to the carbon supply, including new results on it role in regulating carbon-nitrogen interactions. In the last part of the talk I will consider how carbon-signaling regulates key developmental transition like flowering, branching and seed set, which set up a future demand for carbon. The talk will focus on studies with Arabidopsis but I will try to draw parallels with maize.

## Splendor and Misery of Quantitative Genetics in the Era of High-Throughput Technologies

(presenter: Chris-Carolin Schön <chris.schoen@tum.de>)
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Most traits of importance in plant and livestock breeding are regulated by many genes and follow a quantitative distribution. The discipline of quantitative genetics investigates these traits using statistical models with the aim to obtain a more profound understanding of their underlying genetic architecture and to optimize breeding methodology. Quantitative genetic concepts are largely based on summary statistics and predictions rather than on the effect of individual genes and their function. Recent advances in genomic and statistical research have made it possible to incorporate information from high-throughput sequencing, genotyping and metabolic profiling into prediction models and estimation of quantitative genetic parameters. Prediction of the genetic potential of individuals from their DNA sequence has revolutionized breeding schemes by increasing selection intensity and decreasing the length of selection cycles. However, our increase in knowledge on the individual factors underlying quantitative traits is slow despite the ever growing amount of genomic and phenotypic data.
This talk will present methodological developments in quantitative genetics and genomics of maize. Based on genomic, phenotypic and genealogical data from thousands of maize genotypes I will present advancements in genome-based prediction and discuss challenges arising from the high-dimensional nature of genomic information as well as from the large genetic diversity and genome complexity of maize. The effect of integrating knowledge on marker-trait associations from functional or QTL studies and the role of feature selection for improving prediction accuracy will be shown. Factors driving prediction accuracy besides ancestral relatedness will be analyzed for traits of different genetic architecture. Within this context I will discuss quantitative genetic approaches to make best use of the massive collections of genomic and phenotypic data not only for prediction of phenotypes but also to understand the genetic architecture of complex traits and genetic phenomena such as epistasis and pleiotropy.

# McClintock Prize Abstract

**McClintock Prize** **Friday, March 18 7:40PM**

## Horizontal gene transfer – the dark matter of plant genomes and plant evolution

(presenter: Jeff Palmer <jpalmer@indiana.edu>)
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Barbara McClintock's incisive genetic studies unveiled the activity of "jumping genes" in maize and paved the way for current research on the role of transposable elements in gene and genome evolution. Plants serve as superb systems for exploring two other kinds of DNA movement: Intracellular gene transfer (IGT) is the transfer of DNA, including functional genes, among the three genetic compartments of plants, while horizontal gene transfer (HGT) is the non-sexual transfer of DNA between more or less unrelated organisms. I will briefly review some of the major findings from our work on IGT and then focus on recent exciting advances in our understanding of the frequency, mechanisms, and adaptive importance of HGT in the evolution of plants and their genomes. I will also touch on the HGT literature for plant nuclear genomes, an area of notable recent findings. While most of the identified cases involve the acquisition of new genes of apparently major adaptive importance, these are probably the tip of the iceberg of a much larger universe of cryptic, dark-matter HGT affecting many nuclear genes.

Plant mitochondrial genomes acquire foreign DNA via HGT at surprisingly high rates, whereas HGT is unknown in plant chloroplast genomes. Recent work has identified the predominant cellular mechanism – the capacity for native and foreign mitochondria to readily fuse – responsible for mitochondrial HGT. Chloroplasts effectively lack a corresponding mechanism. The capture of foreign mitochondria likely occurs through multiple avenues, including "illegitimate" pollination and direct physical contact between HGT donors and recipients mediated via parasitism, epiphytism, and natural grafting.

Mitochondrial HGT events vary enormously in size, from micro-transfers <100 bp in size to mega-transfers of entire mitochondrial genomes of 50 to hundreds of kb in size. Micro-transfers dominate the HGT landscape of functional mitochondrial genes. Our current studies reveal that these events occur far more frequently than anyone could have imagined, their highly cryptic nature preventing their identification until now. These "dark matter" events occur via HGT followed by or coupled with the gene-conversional replacement of short regions of native genes by foreign sequences. The high frequency of these micro-HGTs leads us to propose that 1) many plant mitochondrial genes have a highly chimeric evolutionary history, 2) much of the sequence variation in plant mitochondrial genes arises not by *de novo* point mutation but via micro-HGT, and 3) these events drive much of the adaptive evolution of plant mitochondrial genomes.

# Short Talk Abstracts

**SESSION 2 – COMPUTATIONAL & LARGE-SCALE BIOLOGY**
Chair: Alain Charcosset Friday, March 18. 8:00 AM – 10:15 AM

**T1**

## Natural variation and cis- regulatory variation for response to abiotic stress in maize

(presenter: Amanda Waters <water157@umn.edu>)
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Plants respond to abiotic stress through a variety of physiological, biochemical, and transcriptional mechanisms. A large number of genes exhibit altered levels of expression in response to abiotic stress, which requires a concerted action of both cis- and trans-regulatory features. In order to study the variability in transcriptome response to abiotic stress, RNA sequencing was performed using 14 day old maize seedlings of inbreds B73, Mo17, Oh43, and PH207 as well as F1 hybrids B73xMo17, Oh43xB73, and B73xPH207 under control, cold, and heat conditions. We identified a large number of differentially expressed genes between parental inbred lines for each condition, as well as many examples of stress-responsive gene expression within each of the inbred lines. By evaluating allele specific transcript abundance in the F1 hybrids we were also able to study natural variation for cis- and trans-regulatory variation between genotypes as well as for stress responsive expression. Although examples of trans-regulatory variation were observed, a large proportion of genes exhibit cis- regulatory variation for both genotype and stress-responsive expression. These genes with allelic variation for cis-regulation in response to cold or heat stress provide an opportunity to study the basis for regulatory diversity. The combined analysis of promoter haplotype diversity (especially at predicted transcription factor binding sites) and stress-responsive expression at these genes in a diverse panel of maize lines revealed potential sources for regulatory variation for maize responses to abiotic stress. An improved understanding of the molecular basis for diversity of gene expression responses to abiotic stress can be used to inform strategies for engineering more stress-tolerant genotypes.

Funding acknowledgement: National Science Foundation (NSF)

**T2**

# Ancient sensing and response networks regulate flooding tolerance – So what's up with maize?

(presenter: Juila Bailey-Serres <serres@ucr.edu>)
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The characterization of the anaerobic polypeptides of maize by Michael Freeling and Marty Sachs in 1980 provided an early demonstration that stress acclimation involves dynamic alteration of gene expression. The synthesis and selective translation of *Alcohol dehydrogenase 1* (*Adh1*) mRNA along with a suite of other transcripts was identified as the core anaerobic response. In the 1990s, a *cis*-regulatory element of the *Adh1* promoter designated the Anaerobic Response Element (ARE) along with *Adh1* untranslated regions were shown to be necessary and sufficient for transcription and translation in oxygen-starved protoplasts, respectively. Fast-forward ten years and the rice Ethylene Response Factor subgroup VII (ERF-VII) transcription factor *SUBMERGENCE1*A was discovered to enable young plants to survive prolonged complete submergence, providing millions of farmers natural flooding insurance. ERF-VII gene families include members that are constitutively synthesized or upregulated by low oxygen and flooding in rice, maize and *Arabidopsis*. The three constitutively synthesized ERF-VIIs of *Arabidopsis* and likely the 14 of B73 maize are destabilized by an oxygen-promoted turnover mechanism. We showed recently that two constitutively synthesized ERF-VIIs of Arabidopsis bind a Hypoxia Response *cis*- Element (HRPE) that has similarity to the ARE of *Adh1* and is present in promoters of genes associated with anaerobic metabolism. This low-oxygen-induced cohort includes several genes encoding proteins that dampen ERF-VII activity or accumulation. RNA-seq analysis of maize seedling and v3 stage plants under control, submerged and recovery conditions, confirmed broad evolutionarily conservation of the hypoxia response gene network including *Adh1*, many of which have predicted HRPEs. Despite the conserved response network, maize seedlings and v3 plants are highly intolerant of submergence. Priming and negative regulation of the hypoxia response gene network may be critical for flooding tolerance. Supported by the US National Science Foundation (MCB-1021969 & Pre-doctoral Fellowship DGE-0813967).

Funding acknowledgement: National Science Foundation (NSF)

**T3**

## Deciphering kernel metabolism: how metabolomics and fluxomic can guide us.

(presenter: Ana Paula Alonso <alonso.19@osu.edu>)
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In view of our rapid growing demands on vegetable oil, enhancing fatty acid synthesis in maize (*Zea mays*) represents tremendous nutritional and economic benefits. In corn kernels, the endosperm and the embryo are the main site for synthesis and accumulation of starch and oil, respectively. So far, breeding efforts to achieve elevated oil content in maize resulted in smaller endosperm and therefore lower yield. We hypothesize that the key to increasing oil content in maize kernels without affecting the yield lies on directly changing their carbon metabolism. To test our hypothesis, we compared the intracellular metabolite levels in maize embryos from two different maize lines, Alex synthetic (Alex) and LH59, which accumulate 48 and 34% of oil, respectively. The comparative metabolomics highlighted the metabolites and pathways that were active in maize embryos and important for oil production. The contribution of each pathway to fatty acid synthesis in terms of carbon, reductant and energy provision was assessed by measuring the carbon flow through the metabolic network ($^{13}$C-Metabolic Flux Analysis) in developing Alex and LH59 embryos to build maps of carbon flow through central metabolism. Our approach combined mathematical modeling with biochemical quantification to identify metabolic bottlenecks in fatty acid synthesis in maize embryos. Transgenic maize plants that we generated to overexpress a lead enzyme increased embryo fatty acid content by 35% without affecting the size of the kernel. This study hence describes the combination of innovative tools that will pave the way for controlling seed composition in important food crops.

**T4**

# Unveiling the Complexity of Maize Transcriptome by Single-molecule Long-read Sequencing

(presenter: Bo Wang <bwang@cshl.edu>)
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*Zea mays* is an important crop species and genetic model for elucidating transcriptional networks in plants. Uncertainties about the complete structure of mRNA transcripts, particularly with respect to alternatively spliced isoforms, limit the progress of research in this system. In this study, we used single-molecule sequencing technology to investigate the maize transcriptome. Intact full-length cDNAs from six tissues of the maize inbred line B73 were barcoded, pooled, size-fractionated (<1 kb, 1–2 kb, 2–3 kb, 3–5 kb, 4–6 kb, and 5–10 kb), and sequenced on the PacBio RS II platform with P6-C4 chemistry. The resultant 111,151 transcripts captured ~70% of the annotated genes in the B73 RefGenV3 assembly. A large proportion of transcripts (57%) represented novel, sometimes tissue-specific, isoforms of known genes, and 3% corresponded to novel gene loci. To validate transcript structures we checked for occurrence of each splice-junction within high-depth Illumina reads generated from matched tissues. Across the tissue sets, on average 90% of splice-junctions were well supported by short-reads. Applied to the PacBio B73 Ref_GenV4 assembly, this full-length cDNA resource has made a large impact on gene annotation, providing evidence to improve many gene models. Furthermore, we identified many novel long non-coding RNAs (lncRNAs) and fusion transcripts. The discovery of many novel transcript isoforms of known protein-coding genes greatly increases our knowledge of the mode, diversity, and complexity of alternative transcript processing. Our observation of tissue-specific patterns of these characteristics suggests that alternative processing operates on wide scale as a means of gene regulation. We also found evidence that alternative processing is influenced by DNA methylation. Our results using single-molecule real-time transcript sequencing show that the maize transcriptome is more complex than previously thought and its characterization far from complete.
This work was funded by NSF grant #1127112 and NSF #1032105.

Funding acknowledgement: National Science Foundation (NSF)

**T5**

# DNA replication timing programs during development of maize root tips

(presenter: Linda Hanley-Bowdoin <linda_hanley-bowdoin@ncsu.edu>)
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Plant cells undergo two types of cell cycles – the mitotic cycle in which DNA replication is coupled to mitosis and the endocycle in which DNA replication occurs in the absence of cell division. We used the thymidine analog EdU and flow cytometry to examine the DNA replication programs of maize root tip cells during the developmental transition from a mitotic cell cycle to an endocycle. B73 root tips were pulse-labeled with EdU, fixed and used for nuclei isolation. Replicating nuclei were fluorescently tagged by conjugating EdU-labeled DNA with Alexa-488. The nuclei were sorted based on DNA content (DAPI) and EdU incorporation into early, mid and late S fractions of the mitotic cycle or the endocycle. Examination of the sorted fractions by 3D deconvolution microscopy identified distinct EdU-labeling patterns during early, mid and late S phase but the patterns were strikingly similar for mitotic and endocycling nuclei in equivalent S fractions. To gain insight into whether the similar patterns reflect conserved replication timing programs, we generated genome-wide profiles of EdU-labeled DNA immunoprecipitated using an Alexa-488 antibody and characterized by Illumina sequencing. Using a novel computational method, we classified the predominant replication time of each locus across the maize genome for both cell cycle types. Comparison of the replication timing profiles revealed that most of the maize genome replicates at the same time during S phase in mitotic and endocycling cells, consistent with the microscopy results. However, over 40,000 regions displayed altered timing, with the regions of altered timing (RATs) representing 11% of the sequenced genome. The RATs are as large as ~200 kb in size but most are <10 kb, and the direction and magnitude of the timing changes differ across the RATs. Future experiments will ask if RATs correlate with structural and/or functional domains of maize chromatin.

Funding acknowledgement: National Science Foundation (NSF)

**T6**

# Genome-wide sequence analysis of the maize FV2 inbred line provides new insights into molecular and chromosomal features of Presence/Absence Variants and their implication in European maize genetic originality

(presenter: Johann Joets <joets@moulon.inra.fr>)
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Maize genetic variation has long been analyzed using molecular or by sequence comparison of selected orthologous regions. These studies revealed an exceptional structural diversity, including copy number variants (CNV) and presence/absence variants (PAV). Analysis of this structural variation has recently been extended at the whole genome scale (1,2). However, maize structural variation has been characterized mainly for American lines and a few Chinese lines, and very little is known about its extent in the European germplasm (3,4,5). To extend the diversity addressed for structural variation, we genome sequenced the French maize FV2 inbred line, which played a key role in European breeding programs over the past 50 years. We generated 90X short-read sequencing of the FV2 genome. Using de novo assembly, we identified More than 10,000 regions that are present in the FV2 genome and absent from the reference B73 genome. These regions cover 87 Mb of genomic sequence, are mainly low-copy and contain 395 new coding-genes. RNAseq analysis of these genes on 12 tissues revealed a potential role in maize adaptation. A new method for PAV-typing was devised and applied to a panel of 25 maize lines. SNPs-based and PAV-based genetic structure analyses exhibited highly consistent results revealing PAVs and SNPs have segregated similarly in this population. Analysis of LD within FV2-specific regions and in flanking regions suggests that PAV sequences have low historical recombination. Our analysis provides new insights into maize genome evolution, structure and function. It highlights how PAVs participated in shaping maize European/American germplasm diversity, and suggests a role of PAVs in maize adaptation.
1 Beló *et al.* (2010) Theor Appl Genet, 120, 355-67.
2 Springer *et al.* (2009) PLoS Genet, 5, e1000734.
3 Swanson-Wagner *et al.* (2010) Genome Res, 20, 1689-99.
4 Lai *et al.* (2010) Nat Genet, 42, 1027-30.
5 Lu *et al.* (2015) Nat Commun, 6, 6914.

Funding acknowledgement: Agence Nationale pour la Recherche, AMAIZING project

**T7**

## Proteomic profiling suggests control of translation and protein stability is crucial for pollen tube germination in maize (*Zea mays*)

(presenter: Johanna Smyth <smythj@science.oregonstate.edu>)
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Germination of the pollen tube in maize occurs rapidly in vitro, with the majority germinating after only 15 minutes. Results from inhibitor studies (e.g., cycloheximide) indicate that de novo translation of polypeptides and protein degradation, rather than activation of transcription, are key facets in the control of pollen tube germination. To test this hypothesis, quantitative profiling of the proteome and phosphoproteome was used to directly assess changes in the transition from mature to germinated pollen. The combined output of three statistical packages in R (IBB, edgeR and PGLEM, FDR of 0.05) identified 393 differentially abundant proteins. Consistent with the inhibitor studies, significant numbers of proteins increase and decrease in abundance (up to ~150-fold) by 30 minutes post-germination. Changes to the proteome were further validated by SDS-PAGE gel silver staining of whole cell protein extracts. Gene ontology analysis revealed several terms associated with translation as significantly enriched among proteins increasing in abundance, further supporting the hypothesis that translation plays a key role in this transition. In addition, 103 proteins are associated with a significant change in phosphorylation state upon germination. These changes are largely independent of change in protein abundance, suggesting that control of protein phosphorylation is also a feature of germination; notably, several kinases and components of the protein degradation machinery show significantly increased phosphorylation. As a genetic test for the biological relevance of the analysis, Ds and Mu insertion mutants with robust PCR genotyping were obtained in 14 genes identified based on the proteomic profiling (e.g, genes that encode proteins that increase in abundance upon germination). Although work is ongoing, both alleles tested to date (a potential transmembrane receptor and a signaling protein) show decreased transmission through the haploid male gametophyte, supporting the idea that this approach identifies cellular components important for pollen function, and possibly pollen tube germination.

Funding acknowledgement: National Science Foundation (NSF)

**T8**

## Identification and characterization of distant enhancers in Zea mays

(presenter: Maike Stam <m.e.stam@uva.nl>)
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Biological processes are tightly regulated and one of the major levels of regulation occurs at the gene transcription level. The fine-tuning of gene expression during e.g. development, differentiation and adaptation to environmental changes is accomplished in multiple ways, including gene regulation by transcriptional enhancers. Unlike promoters, which are located immediately adjacent to the coding regions they regulate, enhancers can be distantly located. This characteristic of enhancers makes it difficult to identify enhancers in a genome. In mammals it has been shown that several epigenetic marks and other chromatin features, such as low DNA methylation, high DNA accessibility and histone acetylation, can be used to identify and distinguish enhancers from the rest of the genome. Enhancers are furthermore characterized by their ability to physically contact their targets via the formation of chromatin loops.
The aim of our study is to identify and characterize distant enhancers in the crop plant Zea mays. Putative regulatory sequences are being identified using published methylC-seq data sets and newly generated DNaseI-seq, ChIP-seq and RNA-seq data sets. Our data indicates the existence of more than 3,000 putative distal enhancers, which also include known and experimentally validated enhancers in maize. We compared two different tissues to identify tissue-specific enhancers and their target genes. A number of the newly identified candidate enhancers will be tested for enhancer function by transient or transgenic expression. In addition, target genes of enhancers will be identified using 4C (Circular Chromosome Conformation Capture).

Funding acknowledgement: European Union - FP7 Marie Curie ITN

**T9**

## Maize genome editing using CRISPR/Cas9 technology

(presenter: Sergei Svitashev <sergei.svitashev@pioneer.com>)
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Targeted DNA double-strand break (DSB) can substantially increase the frequency of genome editing through homology directed repair. In the past decade, several site-directed nucleases have been developed but the recently discovered CRISPR-Cas9 system has truly revolutionized the field due to its simplicity, activity, and versatility. In plants, the ability to generate targeted DSB has three major applications: gene mutagenesis, gene editing, and site-specific gene insertion. Here we report a successful application of CRISPR-Cas9 system as an efficient tool for all three applications in maize. DNA vectors encoding maize codon optimized Streptococcus pyogenes Cas9 endonuclease and guide RNAs, targeting multiple coding and non-coding genomic regions, were introduced into maize immature embryos by biolistic transformation. Mutations were identified at all target sites and plants with bi-allelic multiplex mutations were regenerated. Co-bombardment of CRISPR/Cas9 components with repair DNA templates facilitated native maize gene editing and trait gene insertions. Transient delivery of guide RNA (as RNA molecules) directly into immature embryo cells containing pre-integrated Cas9 also resulted in targeted mutations and ALS2 gene editing. New Cas9 proteins with different PAM recognition sequences substantially expanding the number of potential target sites in maize genome were tested and yielded high frequency of mutations. These results demonstrate the utility of CRISPR-Cas9 technology as a plant genome editing tool to advance plant biology and help meet growing agricultural demands.

**T10**

## Genomes to Fields' Maize GxE Project: Expression of Productivity and Phenological Traits Across a Diverse Set of Environments

(presenter: Elizabeth Lee <lizlee@uoguelph.ca>)
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The Genomes to Fields' Maize GxE Project is testing functional allelic variation across a geographically diverse landscape to evaluate genotype-by-environment interactions (GxE). The intent of this talk is to introduce the scientific community to this ongoing initiative and to illustrate the power of this approach for discovery. In the 2014 growing season, 22 geographically diverse environments were used, ranging from Waterloo, Ontario, Canada to College Station, Texas. A subset of 64 hybrids, part of a larger Maize GxE experiment, were grown across all 22 locations. Agronomic data were collected on grain yield and moisture, days to 50% silking and anthesis, and plant and ear height. Climate data were collected at each location including daily air temperature, daily photosynthetically active radiation (PAR), and daily precipitation. With the geographically diverse locations we were able to elicit a GxE response in a single growing season, however the magnitude of the GxE effect varied across the traits. Using the climate variables we were able to explain a substantial amount of variation in the traits that did not exhibit a large GxE effect. Finally, we illustrate the potential that this approach has for physiological discovery, as we show that accumulated daily light integrals (DLIs) exhibited more predictive power for phenology (i.e., days to silking; $\tau b = 0.82$) than either the CHU ($\tau b = 0.70$) or GDD ($\tau b = 0.45$) thermal time models.

Funding acknowledgement: United States Department of Agriculture (USDA), Iowa Corn Promotion Board, Illinois Corn Marketing Board, Iowa State University's Plant Sciences Institute, National Corn Growers Association, Nebraska Corn Board, Ontario Ministry of Agriculture, Food and Rural Affairs, The Texas Corn Producers Board

**T11**

## Beyond GWAS: Characterizing Drought Response of Elite Temperate and Tropical Maize using a Systems-Biology Approach

(presenter: Addie Thompson <thomp464@purdue.edu>)
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Drought tolerance is becoming an increasingly high-value trait in maize. Due to the difficulty of performing controlled drought field trials, our current understanding the genetics of drought responses is limited. This is particularly true in the context of testcross hybrids representing the diversity of elite temperate and tropical germplasm. Here, recently expired Plant Variety Protection (ex-PVP) inbred lines and Drought Tolerant Maize for Africa (DTMA) inbred lines were crossed to a common tester, PHP02, and grown over two years in controlled-irrigation drought trials in Arizona. Relevant phenotypes (flowering time, plant and ear height, stay-green, yield, and test weight) were collected under both well-watered and drought conditions. Statistical analyses quantified i) the extent of variation present in temperate vs. tropical material, ii) the heritability of traits observed under these treatments, and iii) the phenotypic and genetic correlations between treatments. Traits were then linked to genetic variation via a Genome-Wide Association Study (GWAS) to investigate the basis of drought-affected phenotypes in the temperate and tropical gene pools. Top GWAS hits were subjected to a Weighted Interaction SNP Hub network analysis to identify biological pathways responding to drought and/or corresponding to hybrid performance. Characterization of temperate and tropical germplasm using genome-wide marker effects revealed untapped genetic potential and target haplotype regions for improving drought tolerance in maize.

Funding acknowledgement: The Howard G. Buffett Foundation

**SESSION 4 - GENOME STRUCTURE & CYTOGENETICS**
Chair: Ruairidh Sawers Friday, March 18. 4:40 PM – 6:00 PM

**T12**

## B73 maize population structure analysis by RNA-seq data

(presenter: Zhikai Liang <zliang@huskers.unl.edu>)
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B73 is a maize accession widely used in genetic, genomic, and phenotypic research around the world and also served as the reference for the maize genome sequencing project. The advent of large-scale RNA-sequencing as a method of measuring gene expression presents a unique opportunity to assess the level of relatedness among individuals believed to belong to the B73 maize accession. Using 27 RNA-seq datasets from 20 independent research groups around the world, the level of haplotype conservation and divergence across the genome was assessed. Several clearly distinct clades were identified among putatively B73 samples, which were distinguished by clearly defined blocks of introgressed sequence related to the published reference genome. The largest clade, which is also most closely related to the published reference sequence retained sufficient phylogenetic signal to recapitulate a number of mentor/mentee relationships within the maize genetics community. However, when considering the age of the B73 accession -- greater than 40 years -- and the challenges of maintaining isogenetic lines of a naturally outcrosses species, a strikingly high overall level of conservation was found among B73 samples from around the globe.

Funding acknowledgement: UNL start-up funds

**T13**

# Ecological determinants of transposable element distributions in the B73 maize genome

(presenter: Michelle Stitzer <mcstitzer@ucdavis.edu>)
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Transposable elements (TEs) have incontrovertibly succeeded in the maize genome, where more than 85% of the sequence can be ascribed to past transposition. Evidence from a few loci, such as *bz1* and *adh1*, has shown that in intergenic regions, over time, transposition generates chiefly intact retrotransposons sequentially nestled inside of each other. In contrast, genome assemblies to date have failed to recover these patterns, as repeated sequences complicate assembly, generating a discontinuous collection of TE fragments. The PacBio AGPv4 assembly of B73 provides near-complete genome contiguity, with few gaps. We annotate more than 150,000 full-length TEs based on structural features that originate upon transposition, such as long terminal repeats and target site duplications. In our new annotation, more than two thirds of the genome (~1550 Mb) can be identified as nested and structurally recognizable TEs, and an additional 400 Mb arises from deleted TEs.
An annotation of this scale allows for the first time an investigation into the ecological drivers of TE abundance and distribution. Consistent with previous extrapolations, this diverse genomic ecosystem consists of a few large families of LTR retrotransposons and thousands of smaller families of LTR and DNA transposons. Integrating a variety of data, from gene expression to recombination rates to chromatin state and siRNA abundance, we model the relationship between features of the genomic environment and TE age and TE family abundance. These analyses reveal that individual TE families subsist in the genome using dramatically different strategies of insertion preference, competence for autonomous transposition, and influence on local methylation and gene expression. We conclude that while the impact of transposition is highly family- and context-dependent, a family-level understanding of the ecology of TEs in the genome will refine our ability to predict the role of TEs in generating genetic and phenotypic diversity.

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**T14**

# Resolving the centromere paradox – microevolution of centromeric DNA repeats

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The diversity of centromere-specific DNA repeats within and between species (centromere paradox), seemingly paralleled by the rapid evolution of the cenH3 histone protein, has previously been postulated to be due to evolutionary pressures acting on both molecules based on their interaction (centromere drive hypothesis). Here we show that selection for nearby genes, not the centromere repeats themselves, drives replacement of centromeric DNA.

Teosinte and *Tripsacum* centromeres generally contain large amounts of the tandem DNA repeat CentC. In contrast, modern maize inbreds vary significantly in the amount of CentC at each of the ten centromeres, and many centromeres have lost CentC to the point of being nearly undetectable by fluorescent *in situ* hybridization (FISH). These centromeres tend to be rich in Centromeric Retrotransposons (CR). We combined FISH, cenH3 ChIP-seq data and centromere phylogenies reconstructed from the 25 NAM line parents to follow the changes in centromere repeat composition over time, and document gradual or catastrophic loss of CentC compensated by expansion of the cenH3 domain to flanking, or its relocation to nearby (<4Mb) non-overlapping, regions. These neocentromeres are subsequently colonized by CR elements (about one insertion per century) that were previously shown to give rise to novel tandem repeats. This sequence of events provides a mechanism for the evolutionarily rapid replacement of centromere repeats at genetically nearly indistinguishable loci.

In contrast to most of the genome, only a tiny proportion of teosinte genetic diversity is captured at centromeres of domesticated maize. Seven of ten centromeres are represented by only one or two post-domestication haplotypes. Inbreeding, a consequence of selection for favorable centromere-linked allele combinations, appears to be responsible for the frequently observed disturbance of established centromeres in maize and other crop plants. In nature, genetic bottlenecks may favor the appearance of novel centromere repeat sequences in genetically isolated individuals during speciation.

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**T15**

## Analysis of maize meiotic mutants to fix heterosis

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Meiosis is a crucial step in sexuality that allows new genomic combinations (through recombination) and half the genome size. During meiosis the genome actively remodels itself. Tinkering with meiosis and fertilization in Arabidopsis was successful to switch the reproductive mode from sexual to clonal. We want to valorize the maize non transgenic meiotic mutants collection to form an apomeiotic maize, the first step for an apomictic maize that could propagate heterosis (Ronceret and Vielle-Calzada 2015). Four mutants are necessary for this approach including two meiotic mutants already cloned (AFD1/REC8 and SPO11-1). SPO11-1 is a essential enzyme that introduces DNA double strand breaks (DSBs) to initiate hotspots and crossovers. We have characterized an allelic series of five maize SPO11-1 mutations, by forward and reverse genetics. All the maize *spo11-1* alleles show meiotic defects that mainly lead to asynapsis and univalent formation. Most of *spo11-1* mutants meiocytes show complete absence of DSBs by TUNEL assay and absence of RAD51 foci in the mutant nuclei. However by contrast to what is described in *spo11* mutants in other species, rare meiocytes analyzed show residual signs of recombination leading to one to two bivalents. This data suggests a minor SPO11-1 independent DSB formation pathway in maize. In addition to these early recombination defects, cytogenetical analyses show other chromosomal meiotic abnormalities in *spo11-1* mutants. These data show a link between the initiation of recombination and axial element conformation predicted but never observed in other species.

**T16**

## Identifying the diamond in the rough: Studying allelic variation for complex traits in maize landraces

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Maize landraces are a major reservoir of genetic variation, containing beneficial alleles for improving global agriculture. We report a novel population design appropriate for complex trait dissection in landraces called F-One Association Mapping (FOAM) population. This approach consists on sampling and genotyping at high density single individuals from numerous populations, and from those individuals deriving F1 progeny, which is used for multi-location phenotyping. We constructed a FOAM population from a comprehensive panel of 4,500 landrace accessions from 35 countries in Latin America. Using 1 million Genotyping by Sequencing SNP markers, we infer the presence of genomic structural variation, including Ab10, large chromosomal inversions, and various centromeric alleles. Using the geographic information from the landraces' sampling locations, we map thousands of variants associated with adaptation to environmental characteristics including 711 genes associated with elevation, 1,422 with latitude, 1,202 with longitude, 1,467 with nutrient availability, 958 with annual precipitation and 677 with annual temperature. We observed a significant role of some structural variants in local adaptation, with very significant effect of the adaptive inversion introgression INV4m in high elevation. To further characterize the genetic architecture of complex traits in these landraces, we studied the genetic architecture of flowering time. We show significant association with 14 candidate genes, as well as association at hundreds of genes across the genome. We see a significant overlap between flowering time genes and those involved in local adaptation, including large structural variants, which controlled 30.2% of explainable variance for flowering time. The most significantly associated markers displayed good predictive ability in genome wide prediction. With geographic, genotypic, and phenotypic data publicly available, our landrace FOAM population represents a very powerful resource for the community to study quantitative trait variation and evolution, and offers an excellent framework for allele mining of beneficial alleles.

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**T17**

# Optimal design of genomic prediction in maize hybrid breeding

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Given a set of *n* parental lines, generating and phenotyping all $n^2$-*n* reciprocal hybrids are formidable challenges, when $n > 100$. Although genomic prediction is expected to identify promising hybrids with phenotyping some hybrids in the yield trials, optimal designs are urgently needed in maize commercial breeding for the following reasons: a) to obtain high and stable prediction accuracy, b) to select favorable genotypes with high reliability, and c) to efficiently allocate available resources. Here we propose an optimal design of genomic prediction based on two criteria: connectedness between training and testing populations, and diversity within training population. This new design is illustrated by a diallel date set, comprised of 276 hybrids from 24 maize diverse founders of the nested association mapping population. Our results suggest that this design outperforms other designs in terms of high and stable prediction accuracy with minimal training size. We also show that considering heterotic pattern benefits genomic prediction for grain yield. This research provides a general guideline to design genomic prediction projects in maize hybrid breeding. Building on the study with diallel, we can further conceive optimal designs for hybrid populations generated by other mating designs such as factorial, nested. These optimal designs have the capacity to empower genomic prediction in the context of rapid advancement in genomics and high throughput phenotyping platforms.

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**T18**

# The Effect of Host Genetics on the Maize Leaf Microbiome across 270 Diverse Inbred Lines

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A largely unexplored area in global food security is the interaction between crop plants and the microbial communities around them. The exceptions usually involve either diseases (e.g., rusts and blights) or a few specific symbioses (especially rhizobia and mycorrhiza). The general microbial community—the crop "microbiome"—nonetheless plays an important but poorly understood role in crop health. To identify the influence of host genotype on microbial communities, we collected leaf samples from across ~270 diverse maize inbred lines. The bacterial community of these leaves was determined by targeted 16S amplification and deep sequencing. Bacterial taxa were identified by both standard clustering methods (species-level) and minimum entropy decomposition (subspecies/strain level). Analysis of these data reveal that the maize leaf is a low-diversity community, with most sequence reads mapping to 5-30 species in each sample. Most bacterial taxa have low heritability scores, implying that they are largely determined by the environment. Some taxa, however, show significant heritability. Genome-wide association (GWAS) of these taxa was used to identify the genetic loci influencing their abundance, and these loci were matched against known candidate genes. This is the most diverse maize microbiome study to date, and these results will direct future analyses to identify ways to manipulate the microbiome to improve crop performance.

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**T19**

# Genome-wide Association Study Reveals Genetic Basis and Role of *ZmVPP1* in Drought Tolerance in Maize Seedlings

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Maize production is frequently threatened by drought stress on a global-scale. Identification of the genetic components underlying the drought tolerance in maize is of great importance. To dissect the genetic basis of natural variation of maize drought tolerance, we performed a genome-wide association study (GWAS), by analyzing a natural diversity population, representing a global maize genetic diversity. As a result, 83 loci resolved to 42 candidate genes are identified to contribute to the drought tolerance in maize seedlings, which can explain 55.2% of phenotypic variation. The gene ontology analysis found that 18 genes are linked to a biological pathway involved in plant stress response. Some of them have been characterized to implicate in drought response or tolerance; such as the maize homologues of Arabidopsis ABO1, RCI2A, CYS6, AVP1, SRO1, and ZmNAC111. The other genes are predicted to be involved in transport, development, metabolism etc. The peak signal of GWAS uncovered that the natural variation in ZmVPP1, encoding a vacuolar-type H+-pyrophosphatase, most significantly contributes to maize drought tolerance at the seedling stage. A 366-bp insertion in ZmVPP1 promoter confers drought-inducible expression of ZmVPP1 in tolerant genotypes. Both ZmVPP1 transgenic and allele-introgression strategies can significantly improve maize drought tolerance. Taken together, this research provides important genetic insights into the natural variation of drought tolerance in maize seedlings. The identified loci/genes can be either converted into molecular markers for genetic selection in breeding programs or serve as direct targets in genetic engineering for improvement of drought tolerance in maize.

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**T20**

## BALANCE, a powerful population to decipher complex traits in maize.

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Association study has become a method of choice to identify genomic regions and genes involved in the variation of both qualitative and quantitative traits in many species. Structuration of genetic diversity, especially in maize, is unfortunately detrimental to the identification of true positive associations even if statistical models devised to control the effect of the structure are used. Multiparent Advanced Generation Inter Cross (MAGIC) populations present novel challenges and opportunities for unravelling genetic bases of complex traits and for improving breeding populations.
We present here the MAGIC maize population developed by our group from a 16 way funnel cross of historical lines representatives of the most significant heterotic groups used for hybrid production in temperate regions. Each MAGIC doubled haploid line extracted from the 3th intercrossing round is a unique mosaic of the diversity represented by the parental lines and the whole population contains globally an equal contribution of the founders, linking the resolution of association mapping to the power of linkage analyses. We genotyped and phenotyped a subset of 400 lines chosen to flower in a window of 1.5 weeks and used it to show the general features of the population, and identify candidate genes for complex phenotypes. We sequenced the 16 parental lines and imputed the genome of the HD lines. Simulations of statistical power showed that the population enables efficient QTL mapping, even if its size is rather modest. This size makes manageable precise phenotyping for complex traits in the field (drought, NUE) as well as in controlled conditions experiments.
We will discuss the perspectives given by this population for gene discovery and deciphering complex traits.

**T21**

# *KRN4* Controls Quantitative Variation in Maize Kernel Row Number

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Kernel row number (KRN) is an important component of yield during the domestication and improvement of maize and controlled by quantitative trait loci (QTL). Here, we fine-mapped a major KRN QTL, *KRN4*, which can enhance grain productivity by increasing KRN per ear. We found that a ~3-Kb intergenic region about 60 Kb downstream from the SBP-box gene *Unbranched3* (*UB3*) was responsible for quantitative variation in KRN by regulating the level of *UB3* expression. Within the 3-Kb region, the 1.2-Kb Presence-Absence variant was found to be strongly associated with quantitative variation in KRN in diverse maize inbred lines, and our results suggest that this 1.2-Kb transposon-containing insertion is likely responsible for increased KRN. A previously identified A/G SNP (S35, also known as Ser220Asn) in *UB3* was also found to be significantly associated with KRN in our association-mapping panel. Although no visible genetic effect of S35 alone could be detected in our linkage mapping population, it was found to genetically interact with the 1.2-Kb PAV to modulate KRN. The *KRN4* was under strong selection during maize domestication and the favorable allele for the 1.2-Kb PAV and S35 has been significantly enriched in modern maize improvement process. The favorable haplotype (Hap1) of 1.2-Kb-PAV-S35 was selected during temperate maize improvement, but is still rare in tropical and subtropical maize germplasm. The dissection of the *KRN4* locus improves our understanding of the genetic basis of quantitative variation in complex traits in maize.

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**T22**

## The genetic basis and adaptive significance of transcript abundance differences between two maize inbreds.

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Maize yields have dramatically increased in the past century, yet heterosis has not changed greatly. Instead, breeders have developed distinct groups of high quality, complementary inbred genotypes. These inbred lines express traits through underlying processes defined in part by modular regulation of gene expression. Here, using RNA-Seq data from a population of over 100 B73 x MO17 recombinant inbred lines (RILs), we investigate how networks of gene expression differ within the population and the genetic basis of the differences. We also investigate evidence of lineage-specific selection for different network modules. We find distinct groups of co-expressed genes, or network modules, across the inbred lines. These modules represent key cellular processes. Gene expression abundance of module genes is frequently under strong genetic control. Thus, we identify key regulatory alleles from each parent that control distinct cellular processes. Using Orr's sign test to differentiate selection from genetic drift, we identify a number of network modules that have come under positive selection in one maize lineage. Our results suggest that selection for regulatory alleles has played a critical role in maize inbred breeding.

Funding acknowledgement: NSERC, Genome Canada

**T23**

## Biomolecular interpretation of the Trojan horse myth: Use of *Ustilago maydis* to analyze the function of maize (*Zea mays*) MAC1.

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Plants lack a germ line, thus within the flower adult somatic cells must switch from mitotic proliferation to competence for meiosis. In maize (*Zea mays*) anthers the first step is archesporial (AR) cell specification followed by specification of the somatic niche. The small secreted MAC1 protein (encoded by *multiple archesporial cells 1*) plays a pivotal role in this process. Loss of MAC1 results in excess AR cells, delayed periclinal division of the neighboring Layer2-derived cells which normally develop into the somatic niche, and finally to male sterility. To further study the influence of MAC1 in anther development we were inspired by Homer´s Trojan horse myth and developed a novel system to deliver tagged proteins into maize in a highly localized fashion. A genetically modified version of the biotropic maize pathogen *Ustilago maydis* was used to secrete fluorescently tagged MAC1 into the anther apoplast. Using this Trojan horse approach we rescued the *mac1* mutant phenotype locally, where a fungal hyphae contacted L2-d cells, and thus gained deeper, functional knowledge of MAC1 during somatic layer development. This study also proves that *U. maydis*, which infects all aerial parts of the maize plant, can be used as a valuable tool to characterize maize proteins *in vivo* without genetic modification of the plant. In particular, testing roles of secreted proteins in seedling leaves, adult leaves, and in ears at stages susceptible to *U. maydis* infection should be successful using this system and offers the possibility of rapid testing of predicted secreted maize proteins for *in vivo* functions.

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**T24**

# TANGLED-1 Function in Maize

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The cell wall keeps plant cells fixed in place. Proper orientation of the cell wall during division determines the relative position of the resulting daughter cells. Cell positioning is important for the proper development of a plant: defects may lead to alterations in development and overall growth. Before division a structure named the pre-prophase band (PPB) is formed then disassembled during the transition to metaphase. Later, the phragmoplast guides formation of the new cell wall to the location of the former PPB. Maize *tangled1 (tan1)* mutants exhibit misplaced cell walls and disordered cell patterning. These misplaced cell walls may result from improperly placed PPBs or defects with phragmoplast guidance to the division site. Mathematical modeling using 3D cell shape showed that both wild type and *tan1* mutants properly place PPBs. TAN1 function was analyzed using live cell time-lapse imaging with YFP-TUBULIN. Every completed wild-type division (n=72) was predicted by PPB position. In contrast, *tan1* mutants often had a phragmoplast guidance defect 37.5% (n=34) as well as delays in metaphase and telophase. Therefore, TAN1 function promoted the progression of division and phragmoplast guidance. Full length, native promoter driven TAN1-YFP colocalized with the pre-prophase band and was maintained at the division site throughout division. TAN1-YFP presence after pre-prophase band disassembly suggested that TAN1 promoted phragmoplast growth toward the correct division site. The TAN1-YFP construct rescued both the growth defect and division plane defect of *tan1* mutant. To study the contribution of TAN1 during telophase, a temporally regulated TAN1 construct that is selectively degraded during anaphase was used. This yielded a partial mutant phenotype: a *tan1* mutant expressing this construct grew as well as wild type siblings but had slower division time and minor phragmoplast guidance defects suggesting that division plane orientation and plant growth may be separable functions.

Funding acknowledgement: National Science Foundation (NSF)

**T25**

# Induced and natural variation in genes encoding the microtubule severing ATPase, katanin p60 (*KTN1*), alter meristem shape, plant morphology and spikelet density

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Cortical microtubules (CMTs) facilitate anisotropic growth by templating parallel rings of cellulose microfibrils, via a *KTN1*-dependent mechanism, around elongating cells that restrict the direction in which a cell can expand. We mapped the semi-dominant maize mutant *Clumped tassel1* (*Clt1*) to a *KTN1* homolog on Chr 8 (*Zmktn1a*). Strongly resembling loss-of-function katanin mutants in Arabidopsis, *Clt1* plants show reduced height, smaller organs and increased spikelet density that is accompanied by a wider tassel meristem. Transforming the maize *ktn1a-Clt1* allele into wildtype Arabidopsis causes shorter and wider siliques, and it is unable to complement a *ktn1* mutant. Conversely, the *ktn1a-B73* allele has no effect in wildtype Arabidopsis and can complement *ktn1* mutants. An additional maize nonsense allele, *ktn1a-dcd3*, produces phenotypes reminiscent of *Clt1* homozygotes when combined with *ktn1a-Clt1*.
An enhancer/suppressor screen crossing *Clt1* with the NAM inbred parents revealed *Clt1*/+ plants with a novel phenotype, showing even shorter plants and compressed upper internodes. Unlike the wider tassel meristem and increased spikelet density in *Clt1*, these compressed internodes develop from SAMs that are narrower than normal. In each case, the enhancer mapped to a Chr 3 interval containing *ktn1b*, the homoeolog of *ktn1a*. Consistent with *ktn1b* underlying this modifier, inbreds causing the enhanced phenotype each had independent *ktn1b* alleles with deleterious splicing defects, while inbreds not showing compressed upper internodes produce intact transcripts. Because the phenotypes of the *ktn1a-Clt1* mutant, the *ktn1a-Clt1*/+; *ktn1b-NAM* double mutants and the NAM inbred parents carrying the enhancer alleles are all distinct from each other, we hypothesize that *ktn1a* and *ktn1b* functions have diverged, but remain partially redundant.

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**T26**

# The maize Mid-Completing Activity protein NARROW ODD DWARF is required for normal plant growth and leaf pattering

(presenter: Marisa Rosa <massrosa@berkeley.edu>)
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The establishment of plant architecture is a complex process that relies on the function of multiple genes, acting in various developmental pathways. The shoot apical meristem (SAM) is responsible for the production of above-ground organs and, therefore, regulation of its activity is required for correct plant development. We have identified nod (narrow odd dwarf), a recessive maize mutant with severe pleiotropic developmental phenotypes. NOD is the maize ortholog of the plant-specific MCA (mid-complementing activity) proteins. MCAs are broadly expressed membrane-localized proteins with suggested calcium channel-related activity. They function in development, mechanosensing and calcium absorption in roots in Arabidopsis and rice. Severe developmental defects have only been observed in maize. *nod* mutants have an overall reduction in plant size, due to lower height and narrow, short leaves. Additionally, loss of apical dominance in the B73 background leads to the presence of multiple tillers that give plants a small bushy appearance. Proximal-distal patterning is also affected. The maize leave is normally composed of a proximal sheath and distal blade with the fringe-like ligule and auricle forming a clear border between sheath and blade. In *nod* leaves, the border between sheath and blade is abnormal and ligules are not correctly positioned. This results in the presence of both sheath and auricle cells in the blade portion of leaves. These defects get progressively worse with SAM function. *nod* plants are affected in various other aspects of development including, juvenile to adult transition, stomatal patterning and inflorescence growth and branching. RNA seq analysis of 3 week old shoot apexes shows numerous differentially expressed genes involved in multiple processes, including cell wall biology, hormonal pathways and stress response. This suggests a multi-faceted role of NOD in maize growth and developmental processes.

Funding acknowledgement: National Science Foundation (NSF)

**T27**

# Maize *YABBY* genes *drooping leaf1* and *drooping leaf2* affect agronomic traits by regulating leaf and floral architectures

(presenter: Josh Strable <strable@iastate.edu>)
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Leaf and floral architectures contribute significantly to yield. Leaf architecture, *i.e.*, leaf length, width and angle, influences canopy structure, light penetration and photoassimilate production. In cereals floral architecture defines the floral units that produce grain. A major challenge in plant biology is to understand the genetic factors that regulate leaf and flower development, two disparate but evolutionarily related organs. We discovered and characterized a novel maize mutant we named *drooping leaf* (*drl*), whose acutely flaccid leaves lacked a midrib. In addition to reduced leaf angle, *drl* leaves had slightly shorter, narrower blades and longer sheaths. In pistillate and staminate florets, *drl* mutants displayed ectopic inner-whorl organs. These phenotypes were drastically enhanced by a modifier locus from the Mo17 inbred. We cloned the underlying gene, the *drl1-R* allele, and identified its paralogous enhancer, the *drl2-Mo17* allele, using positional cloning. The genes encode the maize CRABS CLAW co-ortholog, a putative transcriptional regulator with zinc-finger and YABBY domains. Our analysis suggests natural variation in Mo17 at the *drl2* locus enhances the *drl1* mutant phenotypes. Genome wide association studies using maize NAM-RIL populations indicate that the *drl* loci reside within QTL regions for leaf angle, leaf width and internode length, and moreover, identified several rare SNPs with large phenotypic effects for the latter two traits. Expression analyses demonstrate *drl* transcripts accumulate in lateral primordia, but not in vegetative or floral meristems. However, *drl* gene function influences meristem activity, suggesting the *drl* loci likely function non-cell autonomously. Genetic interaction analyses with mutants that affect leaf and floral development reveal a requirement for the *drl* genes early in the establishment of leaf and floral architectures. To our knowledge, this study provides the first evidence of *YABBY* function in maize, and furthermore, illustrates the impact the *drl* genes have on key agronomic traits that influence yield.

Funding acknowledgement: National Science Foundation (NSF)

**SESSION 9 - BIOCHEMICAL & MOLECULAR GENETICS I**
Chair: Petra Wolters Sunday, March 20. 8:20 AM – 9:50 AM

**T28**

## Maize cytolines provide key nuclear genes that are under the control of retrograde signaling pathways in plants

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The genomes of the two plant organelles encode for a relatively small number of proteins. Thus, nuclear genes encode the vast majority of their proteome. Organelle-to-nucleus communication takes place through retrograde signaling (RS) pathways. Four such pathways have been defined for the chloroplast and one for the mitochondrion. Signals relayed through these pathways have an impact on nuclear gene expression (NGE) but their target-genes remain elusive in a normal state of the cell (considering that only mutants and stress have been used to investigate how NGE changes in response to such stimuli). Here we use maize cytolines as an alternative. The nucleus of a donor line was transferred into two other cytoplasmic environments through at least nine back-crosses, creating three cytolines (a.k.a., isonuclear lines). Their transcriptomes were sequenced and compared. There are 96 nuclear genes that are differentially regulated in the two cytoplasm donor lines when compared to the nucleus donor. They are expressed throughout plant development, in various tissues and organs. One third of the 96 proteins have a human homolog, stressing their potential role in mitochondrial RS, rather than chloroplast. An analysis of the promoter region of the 96 genes revealed that six harbor more than 10 binding sites for ABI4, a known transcription factor that integrates signals from three of the four plastid RS pathways, in addition to the one defined for mitochondria. We also identified syntenic orthologous genes in four other grasses and orthologous genes in Arabidopsis thaliana, hinting towards a general mechanism in plants, where the RS pathways target these nuclear genes. More importantly, the 96 genes are not differentially regulated as a result of mutation, or any kind of stress. They are rather key players of the organelle-to-nucleus communication in a normal state of the cell.

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**T29**

# The maize *dosage-effect defective kernel1* (*ded1*) locus encodes a MYB transcription factor controlling endosperm development and grain-fill.

(presenter: Janaki Mudunkothge <jmudunkothge@ufl.edu>)
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Grain yield and seed weight are important target traits for the improvement of seed crops. To identify genes that control maize seed weight, we screened 1,000 defective kernel mutations from the UniformMu transposon-tagging population for seed dosage-effects using individual seed weight and single-kernel near infrared reflectance spectroscopy. The *ded1* mutant was identified in this screen as showing segregation distortion when normal kernels are separated by seed weight. Homozygous *ded1* mutant seeds arrest embryo development and the basal endosperm transfer layer (BETL) fails to differentiate. Mature *ded1* kernels have reduced grain fill and only 15% of these seeds germinate. The *ded1* locus was mapped to the long arm of chromosome 1. The 358 kb mapping interval contains a novel retrotransposon insertion within a MYB domain transcription factor locus. The insertion is not found in the progenitor W22 haplotype and the insertion disrupts the MYB gene open reading frame. We created new alleles in this candidate gene through CRISPR/Cas9 targeted mutagenesis. Preliminary data shows that the mutant alleles fail to complement the *ded1* reference allele indicating molecular cloning of the *ded1* locus. We validated published transcriptomics data that shows the *Ded1* gene is expressed specifically in developing embryo and endosperm and shows paternal biased expression. In agreement with paternal biased expression, *ded1* heterozygous seeds show reduced seed weight when the mutant allele is transmitted through pollen. These data indicate that paternal expression of *Ded1* promotes grain-fill and are consistent with the parental conflict hypothesis for the selection of imprinted genes in angiosperms.

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

**T30**

# Maize *white seedling 3* results from disruption of homogentisate solanesyl transferase

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Maize *white seedling 3* (*w3*) has served as a model albino-seedling mutant since its discovery in 1923. We show here that the *w3* phenotype is caused by disruptions in homogentisate solanesyl transferase (HST), an enzyme that catalyzes the committed step in plastoquinone-9 (PQ9) biosynthesis. This reaction lies at the heart of a complex metabolic network of plastidial antioxidants, pigments, and phytohormones. Disruption of PQ9 biosynthesis has broad-ranging implications, from nutritional quality of grains to plant defense. Plastoquinone-9 is a redox cofactor required for electron transfer and proton translocation during photosynthesis. It also serves as an oxidant in the enzymatic desaturation of phytoene during the formation of carotenoids. Finally, plastoquinone-9 is the immediate precursor for plastochromanol-8 (PC8), a vitamin E analog with roles as a lipid-soluble antioxidant. As observed in corresponding Arabidopsis mutants, plastoquinone-9 deficiency resulted in albino seedlings defective in carotenoid biosynthesis at the level of phytoene desaturation, and accumulation of phytoene. Unlike in Arabidopsis, maize mutants showed a propensity for vivipary, typical of abscisic acid-deficient mutants, again resulting from carotenoid deficiency. In addition, the non-green seeds of maize provided a unique opportunity to examine the effects of plastoquinone deficiency on tocochromanol (tocopherol, tocotrienol, and PC8) accumulation in the absence of photo-oxidation. As in leaves, the absence of HST in seeds resulted in loss of PQ9, PC8, and carotenoids as well as the accumulation of phytoene. However, tocochromanol content in *w3* kernels remained similar, supporting the hypothesis that HST's influence on vitamin E levels was not due to a biosynthetic role per se, but rather to its protection of pigments (including chlorophyll and tocochromanols) in green tissues from photo-oxidative damage.

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

**T31**

# Autophagic Recycling plays a central role in maize nutrient remobilization

(presenter: Richard Vierstra <rdvierstra@wustl.edu>)
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Autophagy is a primary route for nutrient recycling in plants by which superfluous/damaged cytoplasmic material and organelles are encapsulated into double membrane-bound vesicles and delivered to the vacuole for breakdown. Central to autophagy is a conjugation system that attaches phosphatidylethanolamine to AUTOPHAGY-RELATED (ATG)-8, which then coats emerging autophagic membranes and helps promote cargo recruitment, vesicle enclose, and subsequent vesicle docking with the tonoplast. A key component in ATG8 lipidation is ATG12 that provides the ligase activity upon its covalent attachment to ATG5 and subsequent assembly into an ATG12-ATG5/ATG16 complex. Here, we defined the maize ATG system by transcriptome analyses and characterized it genetically through *ZmAtg12* mutants. RNA-seq analysis revealed that the expression of most ATG components are significantly increased in senescing leaves/tissues and in endosperm, suggesting specific role(s) in these organs. Two UniformMu insertion mutants were identified in *ZmAtg12* that delete the C-terminal end of the protein and thus block its attachment to ATG5. Mutant plants have compromised autophagy as determined by localization of the YFP-ATG8a reporter and its vacuolar cleavage during nitrogen or fixed-carbon starvation. *Zmatg12* plants are phenotypically normal and fertile when grow under nitrogen-rich conditions. However, when nitrogen starved, growth is severely retarded, and as the plants mature, they show enhanced leaf senescence and delayed ear development. Nitrogen partitioning studies revealed that remobilization is significantly impaired, which decreases seed nitrogen content and yield. Further metabolome profiling revealed changes (mutant versus WT) in amino acid and sugar content under both nutrient rich conditions and during fixed-carbon/nitrogen starvation, increased ABA levels, and strong alterations in lipid composition consistent with disregulated lipid oxidation and membrane turnover. Together, our studies demonstrate that ATG8-mediated autophagy is not essential to maize, but becomes critical during nutrient starvation by helping promote nutrient/membrane recycling, and thus should severely impacts crop productivity under suboptimal field conditions.

Funding acknowledgement: National Science Foundation (NSF)

**T32**

## Forward genetics identifies the nuclear pore complex component, *aladin1*, as necessary for tassel architecture and asymmetric cell division in maize

(presenter: Norman Best <nbbest@purdue.edu>)
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The nuclear pore complex (NPC) enables and regulates the movement of macromolecules between the nucleus and cytoplasm but also acts as a point of contact between the nuclear compartment and cytoskeleton. The *aladin1* mutant was identified in a B73-background EMS population of maize with shortened upper internodes, upright leaves, altered tassel architecture, and abnormal asymmetric division of stomatal subsidiary cells. Using BSA and high throughput sequencing, we identified a SNP resulting in a nonsense mutation in the last exon and truncation of the last 16 amino acids of the nuclear pore complex core subunit, *aladin1*, as the cause of these phenotypes. A second allele, *aladin1-2*, which encodes a nonsense mutation in the 10th exon was recovered from a targeted mutagenesis screen of ~10,000 EMS-treated M1 plants and confirmed the identity of the gene. Mutation of this gene in humans' results in dysfunction of the central nervous system and triple A syndrome. Analysis of differential mRNA accumulation in the *aladin1-1* mutant compared to WT identified up-regulation of other NPC components in the mutant but no difference in ALADIN1 transcript abundance. The *aladin1-1* mutant was crossed to 25 different inbreds and the phenotype observed in F2. Dramatic enhancement of the mutant phenotype in the M37W background resulted in variably penetrant lethality and up to 90% reduction of plant height among the survivors. The ability of genetic background to affect phenotype expression was matched by environmental lability as *aladin1-1* mutant phenotypes were variably suppressed when grown in the greenhouse. These findings demonstrate that the *aladin1* gene is necessary for normal plant development, shoot architecture, cell division, and synthetically lethal in combination with standing variation in maize.

Funding acknowledgement: National Science Foundation (NSF)

**T33**

# Altered expression of the maize cytochrome P450 CYP78A1/KLUH increases biomass and seed yield by an extended duration of cell division

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Maize is the highest yielding cereal crop, that is grown worldwide for grain yield or silage. We modulated the highly specific expression pattern of the growth enhancing ZmKLUH gene, encoding a cytochrome P450 (CYP78), by using a growth zone specific GA2-oxidase promoter. This resulted in increased organ growth, which in turn gave rise to enhanced seedling vigor and a higher stover biomass and seed yield. The engineered trait was robust as it improved yields in an inbred background as well as in a panel of hybrids, at several locations and over multiple seasons in the field. To unravel how ZmKLUH promotes organ size, the maize leaf was further examined. Transcriptome studies and hormone measurements showed that ZmKLUH functions through an increase in auxin, of which the accumulation pattern in the leaf growth zone is highly similar to the ZmKLUH expression pattern. Detailed analysis of growth over time demonstrated that ZmKLUH stimulates the duration of leaf elongation by maintaining the dividing cells for a longer period in a proliferative, undifferentiated state. This ZmKLUH mediated process is independent of the enhanced leaf growth that is observed by the overproduction of bioactive gibberellins that affects growth rate or leaf elongation rate, as combining ectopic KLUH and GA20-oxidase overexpression results in additive phenotypes. Furthermore, we demonstrate that the prolonged duration of growth serves as a compensation mechanism to maintain the growth potential when plants experience a growth rate reduction caused by abiotic stresses, such as mild drought or cold nights.

**T34**

# Importance of mesocotyl and plumule growth on heat and drought avoidance in modern maize hybrids: physiology and GWAS

(presenter: Jorge Nieto-Sotelo <jorge.nieto@ib.unam.mx>)
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Throughout its improvement history, traditional farming practices for maize production allowed its adaptation to many local environments. In arid and semiarid regions of Mexico and the US southwest, traditional maize farming is managed by combining: i) soils with high humidity beneath the surface, ii) sowing at 20-40 cm depth, iii) local varieties with deep planting (DP) resistance, as most maize landraces and modern hybrids lack this capacity. Here, we studied 284 DTMA (Drought Tolerant Maize for Africa) maize hybrids developed by CIMMYT. In seedling assays, performed in growth rooms in the dark, emergence after DP positively correlated with: a) final mesocotyl length, b) final plumule length. In experimental field trials designed to asess drought stress, DTMA plants that emerged after DP (34 cm depth) displayed lower leaf temperatures and delayed leaf senescence, while the growing degree days for both male and female flowering increased, compared to 5 cm planting. These data indicate that DP promoted drought stress avoidance. Furthermore, under drought, DP increased ear weight, lenght, and width, as well as total grain/ear and 100 grain weight relative to shallow planting. However, compared to DP resistant landraces, DTMAs emerged poorly after DP, indicating that emergence needs to be improved in DTMAs to take full advantage of DP. The phenotypic variation within the DTMA collection allowed us to perform GWAS to identify SNPs associated with mesocotyl lenght. Twenty SNPs accounted for the strongest phenotypic effects (10 positive and 10 negative). Most associated genes are involved in plant growth and development encoding proteins related to DNA binding, small molecule transport, and cell adhesion, among others. Validated genes associated to mesocotyl elongation will allow a better understanding of the genetic network involved in the evolution of DP resistance and the improvement of high yielding maize hybrids better adapted to sustainable agricultural practices.

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# Poster Abstracts

**P1**

## New Trait Data at MaizeGDB

(submitted by Mary Schaeffer <mary.schaeffer@ars.usda.gov>)
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MaizeGDB has several ways to archive trait data used for QTL and GWAS analyses. The simplest is simple posting of files provided by researchers along with links to the publication. More recently we have begun to integrate these data for diversity recombinant germplasm, and association panels. The goal is to integrate these data at one location, so that they may be used with minimal processing by researchers.
Access to the data is provided under the 'Diversity' button on the home page, and, new this year, on individual Stock and Trait pages. For germplasm distributed by the North Central Regional Plant Introduction Station, individual Stock records link to the corresponding GRIN record, from which the germplasm may be requested. Last year our focus was on NAM and IBM mapping population, where we added values for some 60 traits. this year we have integrated published data for two diversity panels: the expanded Goodman panel, (Flint-Garcia et al 2005 Plant J 44:1054-64); and the Ames panel (Romay et al 2013 Genome Biol 14:R55) for many of the same traits. Additional trait categories this year include root architecture; disease response; and shoot apical meristem architecture. All traits are linked to controlled vocabularies, notably trait ontology, and the plant ontology (anatomy, growth), which permit filtering datasets, e.g. all ear traits; and promote interoperability with other crop and model plant species data.

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

**P2**

## MaizeGDB: New Tools and Resource

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

Funding acknowledgement: United States Department of Agriculture (USDA)

**P3**

## MaizeGDB Video Tutorials, Feedback Booth and Introducing the AgBioData Working Group

(submitted by Lisa Harper <lisaharper@me.com>)
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As datasets get larger and more complex, it becomes more difficult for MaizeGDB users to be aware of all the tools and services MaizeGDB provides. In order to help our users, we have made a YouTube channel (https://www.youtube.com/channel/UClV7hOrmTtWjB6fgo_gT_dg) where we are loading all new, short, tutorial videos. We welcome suggestions for new videos! Another aspect of large and complex datasets, is that a degree of standardization between different databases would facilitate greater interoperability and ease of use for the users. Towards that end, MaizeGDB has spearheaded the formation of a Working Group comprised of >110 people who work at >30 agricultural-related database. This group has been meeting for a year, and has several specific objectives, all designed to ultimately make life easier for you, the database user. To help make science results more reproducible and accessible, many groups, including MaizeGDB, will soon require appropriate and standardized metadata (data about the data) for datasets taken in at MaizeGDB. Don't be frightened! This will help YOU in the long run. Lastly, at this poster, there will be a place where you can provide feedback about MaizeGDB. We welcome your comments and suggestions!

Funding acknowledgement: United States Department of Agriculture (USDA)

**P4**

## Breeder Survey, Tools, and Resources to Visualize Diversity and Pedigree Relationships at MaizeGDB

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In collaboration with maize researchers, the MaizeGDB Team prepared a survey to identify breeder needs for visualizing pedigrees, diversity data, and haplotypes, and distributed it to the maize community on behalf of the Maize Genetics Executive Committee (Summer 2015). We received 48 responses from researchers, of which more than half were self-identified as breeders. The researchers established their top priorities for visualization as: 1) SNPs in a region for a given list of lines, 2) haplotype analysis in a given list of lines, and 3) pedigree relationships. The survey identified the following two populations as the most beneficial to visualize: 1) 3000 inbred lines as described in Romay et al. (Genome Biol, 14:R55, 2013), and 2) Expired PVP lines (Plant Variety Protection Act). Driven in part by this stakeholder input, MaizeGDB are currently working in four areas: 1) Displaying immediate progenies of current stocks at the MaizeGDB Stock pages, 2) Curating the most recent ex-PVP lines listed in GRIN into MaizeGDB and displaying them on the MaizeGDB Stock pages, 3) Developing network views of pedigree relationships, and 4) Visualizing genotypes from diversity datasets.

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

**P5**

## Stewardship of the Maize B73 Reference Genome Assembly

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The release of version 4 of the B73 reference genome assembly is imminent. However, continued improvement of the assembly is likely to fall to the maize research community. Toward this end, and recognizing the importance of an accurate and well-curated reference genome, MaizeGDB, Gramene, and the Genome Reference Consortium (GRC) are working together to provide tools for continued improvement of the genome. These tools include: a collection device for assembly and gene model issues reported by the community, available at MaizeGDB; the GRC database and tools for resolving issues and making "patch releases" (releases of the assembly which include corrections and new sequence, but which do not change chromosome coordinates), and eventual full releases when enough issues have been resolved to merit a new version. MaizeGDB is committed to continued stewardship of the genome and will work with researchers to resolve issues.

Researchers are encouraged to report issues they have found in v3 as well as issues found in v4. The v3 will be tested against v4 to see which issues are resolved in the new assembly and which remain to be fixed.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P6**

## Data Management and Analysis Solutions for Maize Predictive Phenomics: A partnership with the GxE Subgroup of the Genomes to Fields (G2F) Initiative

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Breeding decisions to develop improved cultivars for industrial and agricultural uses are greatly facilitated by simultaneously leveraging phenotypic, genotypic, weather, and image data. Development of standardized data collection and analysis practices by working directly with the data generators in the initial stages of the data collection process, is an important first step to support a multi-institutional, multi-year breeding projects. The Genotype by Environment (GXE) subproject within the maize Genomes to Fields (G2F) is a multi-institutional project spanning 25 North American locations. The GXE group is collectively addressing environmental effects on the performance of a large collection of maize inbreds and hybrids grown in 25 diverse locations. Each location is collecting data on 14 core phenotypic traits, as well as weather measurements with image data for a subset of locations. To assist in the management of these diverse data types, we are developing and deploying a robust, yet flexible, data management and analysis platform that meets their immediate needs but is also extendable to the broader plant breeding community. In this poster, we present progress made over the past year working with partners at the iPlant Collaborative and the Breeding Management System software development team at CIMMYT, as well as plans for next steps in 2016.

Funding acknowledgement: National Science Foundation (NSF), Iowa Corn, National Corn Growers, Iowa State University

**P7**

# A dramatically improved maize B73 reference genome constructed using single-molecule technologies

(submitted by Yinping Jiao <yjiao@cshl.edu>)

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A complete and accurate reference genome is imperative for sustained progress in understanding the genetic basis of trait variation and crop improvement in maize. Although the current B73 reference sequence has seen incremental improvements in quality over the last several years, many gaps and misoriented contigs remain due to the complexity of maize genome. To remedy this, we employed Single Molecule Real-Time sequencing technology (PacBio) and NanoChannel Array from BioNano to build the next generation maize reference genome. The de novo assembly of 65X PacBio long reads reached an N50 of 1Mb with 2,908 contigs. With the BioNano genome map, the contigs were scaffolded into 625 hybrid scaffolds with an N50 of 9.6Mb. Using the B73 physical map and IBM genetic map we were able to place 96% of contigs and 99% of the sequence into chromosome-level scaffolds. Polishing the pseudomolecules by gap filling with PacBio reads and base correction with about 100X Illumina data closed a total of 170 gaps and corrected about 80 thousand bases. The number of gaps in the final V4 assembly was reduced from 124,337 in V3 to 2,523, and size estimates for 1,111 of these gaps were obtained from the BioNano map. Total assembly size relative to V3 increased by about 50Mb with most of order and orientation errors corrected. In this new assembly, about 60% of transposable elements (TE) are intact, enabling analysis of TEs diversification as well as improved annotation. In the centromere region, we also saw good overall improvement relative to the v3 maize reference genome. To ensure a comprehensive gene annotation, PacBio Iso-seq technology was used to capture full-length cDNA in six tissues. More details of this update will be presented at the meeting.

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

**P8**

## A maize and its wild relative (*Zea.mays.Mexicana*) genomes provide new insights for domestication, improvement and introgression

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Here we reported two draft genomes for Mo17, a modern and widely used maize inbred and *Zea.mays.Mexicana* (hereafter, *Mexicana*), the most related wild relative of cultivated maize, assembled using a novel meta-assembly strategy based on a distinctive genetic design. The final assembled genomes were 2.04 Gb for Mo17 and 1.20 Gb for *Mexicana* with N50 size for scaffolds of 3Mb and 106Kb, respectively. Comparative analyses reveal the high level of variants diversity between Mo17, B73 and *Mexicana* including mega-based sized structure variations and identify hundreds of genes under positive selection. The mutation rate was calculated directly as 1.17E-07 per site per year in maize based on genome wide sequence data. *Mexicana* genome contributed significantly to maize adaptation and improvement that 6.85% and 5.90% putative introgression regions were identified in Mo17 and B73 genomes respectively and many QTLs were also identified affecting many agronomic important traits. These two genomes offer a valuable resource for the pan-genome construction of *Zea.mays* thus enhancing maize breeding.

Funding acknowledgement: National Hi-Tech Research and Development Program of China (863),the National Natural Science Foundation of China (NSFC)

**P9**

## A new view of the maize genome that incorporates multiple denovo genome assemblies

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Next Generation sequencing technologies have opened the door to multiple genome analyses and an increased understanding of the variation present in the genomes of plants. To date, most of the germplasm analyses have relied on the comparison of sequence reads to a reference assembly of a representative accession limiting our understanding of genome variation to SNPs and small indels. With the recent development of new and faster technologies for denovo sequence assembly, including NRGene's DeNovo Magic assembly, we are now able to cost effectively produce physical maps from many diverse lines of a single species such as maize. To fully analyze multiple assemblies, a new all-by-all comparison approach is needed. GenoMagic software has been developed to handle such analyses. This not only opens the door to understanding the full breadth of variation complexity within species but can also accelerate breeding, marker aided selection and gene discovery. This talk will introduce this comparative analysis approach and show some maize specific examples.

**P10**

## A storehouse of immeasurable worth: Breathing new life into archived GBS data

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While the number of sequence read archive (SRA) files containing raw FASTQ sequences generated in order to make genotype calls using genotyping-by-sequencing (GBS) continues to increase, there remain no convenient means by which to access this abundance of data for other uses outside the original study. In order to address this issue, a pipeline is under development to provide the means to extract specific inbred lines or samples from a larger study for ancillary investigation. This pipeline will allow the user to readily convert SRA files to actionable FASTA sequence files for use in various downstream analyses. As a proof of concept, 79 inbred popcorn lines from the Ames Diversity Panel (ADP) published by Romay et al. (2013) were extracted from the publicly available SRA files and run through the SRAtoFASTA pipeline followed by the conversion of each of the individual popcorn line FASTA files to general feature format (GFF) files for upload to MaizeGDB. The resulting outputs were incorporated into an ongoing study to identify the genetic components of gametophytic incompatibility in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P11**

## A thermo-align approach for the design of template-specific hybridization and priming oligonucleotides for repetitive genomes

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Re-sequencing of target sites in a genome is a widely used technique in modern genomics. Identifying template specific primers and hybridization oligos is critical to the success of these applications. A combined thermodynamic and sequence alignment based computational tool (*thermo-align*) for the identification of template specific primers/hybridization oligos has been developed, which is particularly relevant for studies on organisms with repetitive genomes such as maize. During the initial phase of the pipeline, all possible primers of user-defined ranges in size, GC content and melting temperature are extracted for a given genomic locus. *Thermo-align* also uses available polymorphism information to design oligos that would work among diverse samples of the same organism. An enhanced BLAST-based local alignment algorithm is then used to evaluate the uniqueness of each candidate sequence with respect to the reference genome. The algorithm uses an end-filling approach to obtain full-length alignments in the presence of mismatches, to allow for exhaustive evaluation of potential mis-priming/hybridization sites. A nearest-neighbor thermodynamics model is used to compute energy metrics corresponding to DNA duplex hybridization and secondary structure formation under user defined reaction conditions. In this talk, I will introduce how our *thermo-align* approach addresses some of the challenges to amplify/re-sequence target regions in repetitive genomes. Results will be presented from testing the pipeline for target-specific amplification in maize, which has a highly repetitive genome. This automated *thermo-align* tool is expected to facilitate identification of template specific primers and hybridization oligos for a variety of applications in genomics.

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**P12**

## Analysis of the *zein* gene family in maize W22 inbred line using single-molecule real-time sequencing technology

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Single-molecule real-time (SMRT) sequencing (Pacific Biosciences) produces long and unbiased sequences, which enables the assembly of repetitive regions. Now, we used this method to sequence the W22 inbred line, a Non-Stiff Stalk maize line. In order to investigate how a multigene family has shaped the architecture of the maize genome in a haplotype-specific manner, we focused the analysis on the *zein* gene family, the main component of maize storage protein genes. Compared with B73, a Stiff Stalk line derived from a different breeding experiment, there are copy number variations (duplication, deletion) between these two inbreds. The 27-kD γ-zeins, 10-kD δ-zeins and wild-type allele of the *floury-2* (*Floury-2*, 22-kD α-zein) were duplicated in tandem in W22. Combined with our full-length cDNA library from W22, 16 of 19-kD α zeins and 14 of the 22-kD α zeins appear to be expressed. All the β-, γ- and δ-zeins except 18-kD δ-zein are expressed in W22. The expressed genes, some with premature stop codon, are interspersed with non-expressed genes. Our results revealed the importance of long read-length in determining the complexity of tandem duplicated regions like *zein* gene family clusters. Therefore, the SMRT sequencing technology appeared as an inexpensive way to study copy number variation in complex genomes.

Funding acknowledgement: Waksman Institute of Microbiology

**P13**

## Ascertaining the Co-Expression Networks of Homeobox Genes in Maize Shoot Apical Meristem

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The Shoot Apical Meristem (SAM), from which all aboveground tissues of plants are derived, is critical to plant morphology and development. SAM initiation and development are characterized by distinct transcriptional variation. In maize, loss-of-function mutant studies have identified several well-known SAM related genes, which show distinct SAM-specific expression patterns and act at the top of a hierarchical network including hundreds of genes. Some of these cloned SAM related genes are homeobox genes encoding a typical DNA-binding domain of 60 amino acids, known as a homeodomain, which characterizes a large family of transcription factors. To date, homeobox gene regulatory networks are not well described. Here, we've collected a comprehensive transcriptome dataset of 70 different tissues/stages from maize reference inbred-B73, which includes 12 SAM domains of 2-week seedlings and 6 SAMs of different developmental stages. We constructed a co-expression network of 25,351 genes and identified 1,945 SAM-specific expressed genes. Meanwhile, we uncovered 143 homeobox genes using an exhaustive genome-wide search in maize. Examining the co-expression networks, we observed that 104 homeobox genes are involved in 58 different modules. In addition, 34 homeobox genes exclusively from 3 out of 12 homeobox phylogenetic clades are enriched in SAM-specific expression pattern, show strong functional redundancy in the regulation of transcription, auxin response and transport but diverge in the regulation of phytohormone biosynthesis, cell fate, cell cycle, ubiquitination and other plant growth regulating factors in the co-expression regulatory networks. We've also conducted ChIP-Seq on SAM-specific homeobox genes, of which the analyzing is ongoing. Our results provide a comprehensive co-expression regulatory network of homeobox genes with respect to SAM development.

Funding acknowledgement: National Science Foundation (NSF)

**P14**

## Assembling the genome and transcriptome of maize's sister genus Tripsacum as a step toward identifying freezing tolerance genes

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Freezing temperature poses a severe challenge to many biochemical and physiological processes in plants. The expression of freezing tolerance genes increases during periods of low, non-freezing temperature exposure in a process called cold acclimation. Grass species in the two genera *Tripsacum* and *Zea* differ in their ability to withstand freezing temperatures. Even though both grass genera originated in the tropics, *Tripsacum* species are perennial and can overwinter in a dormant state, while maize cannot survive prolonged freezes. The *Tripsacum* genus diverged from the *Zea* genus fewer than 1.2 million years ago, prior to the domestication of maize (Ross-Ibarra *et al.* , 2009). *Tripsacum* and maize share most of their gene content, yet the basis for freezing tolerance in *Tripsacum* and freezing sensitivity in maize remains largely unknown. Furthermore, *Tripsacum* lacks the genomic resources available for model organisms. To enable mapping of freezing tolerance QTL, we sequenced the *Tripsacum* genome and transcriptome. The *Tripsacum dactyloides* "Pete" cultivar genome was sequenced on the HiSeq2500 platform to produce 379 million 2 x 250 bp reads. The *Tripsacum* genome assembly was guided by the maize B73 reference genome. mRNA from root, crown, leaf, and inflorescence tissue were sequenced on the NextSeq500 platform to produce 2 x 150 bp reads. The *Tripsacum dactyloides* "Pete" and *Tripsacum floridanum* transcriptomes were *de novo* assembled from 56 Gbp and 93 Gbp of sequence data, respectively, using Trinity. Contaminant contigs from other species were filtered out of the genome and transcriptome assemblies based on percent GC content, coverage depth, and taxon alignment. The genome and transcriptome assemblies were evaluated for completeness using the set of Benchmarking Universal Single-Copy Orthologs (BUSCO).

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

**P15**

## Automatic imaging and 3D timeseries analysis of growing roots with shinyDR

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Root system architecture (RSA) has a very important role in plant growth and productivity. Understanding how root systems grow and respond to diverse environments will help to improve plant performance and crop production. Image-based phenotyping technologies enable high-throughput and non-destructive measurements of plant traits. To quantify root system architecture and development, we have developed an automatic optical imaging system that allows three dimensional monitoring of root growth processes over time. Plants of two maize genotypes, B73 and Mo17, were imaged every 4 hours for a week, starting at 2 days after germination. Time-series of three dimensional root shapes were reconstructed using our RSA-Gia pipeline and then analyzed using DynamicRoots software. DynamicRoots software offers automatic computation of structural and dynamic traits of each branch in the root system. We developed shinyDR, an application in R for interactive analysis and visualization of data from DynamicRoots. Using the output files generated by DynamicRoots, shinyDR computes overall root morphological traits, growth rates, root growth directions and branching pattern, and lateral root distribution. Reactive plots allow the user to interactively view the growth process of selected root branches, the dynamics of traits for individual branches during observation time, the volume, length, and radius distribution of lateral roots by branching order at every observation time, the radial angle and branching angle distribution of lateral roots by branching order, among other traits. We demonstrate the shinyDR software and our preliminary work analyzing of the differences in dynamic branching patterns between B73 and Mo17, which will ultimately allow us to model root growth and root-environment interactions with high spatiotemporal resolution.

**P16**

## Bulked Segregant - genotyping-by-sequencing: Cost-effective and background independent genetic mapping of mutants and QTL

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

Funding acknowledgement: National Science Foundation (NSF)

**P17**

## $C_4$ gene discovery using cross species selection scan

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$C_4$ photosynthesis is perhaps one of the best examples of convergent adaptive evolution with over 25 independent origins in the grasses (Poaceae) alone. The availability of high quality grass genome sequence presents new opportunities to explore the mechanisms underlying this complex trait using evolutionary biology-based approaches. In this study, we performed genome-wide cross species selection scans in $C_4$ lineages to facilitate $C_4$ gene discovery. It was enabled by the well conserved collinearity of grass genomes and the recently sequenced genome of a $C_3$ panicoid grass *Dichanthelium oligosanthes*. This method, in contrast to previous studies, does not rely on any a priori knowledge of the genes that contribute to biochemical or anatomical innovations associated with $C_4$ photosynthesis. We identified a list of 88 candidate genes that include both known and potentially novel components of the $C_4$ carbon shuttle pathways. This set includes the carbon shuttle enzymes PPDK, PEPC and NADP-ME as well as several predicted transporter proteins that likely play an essential role in promoting the flux of metabolites between the bundle sheath and mesophyll cells. Importantly, this approach demonstrates the application of fundamental molecular evolution principles to dissect the genetic basis of a complex photosynthetic adaptation in plants. Furthermore, we demonstrate how the output of the selection scans can be combined with expression data to provide additional power to prioritize candidate gene lists and suggest novel opportunities for pathway engineering.

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

**P18**

## Camoco: systematic integration of co-expression and genome wide associations studies in Zea mays to detect causal variants.

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Genome wide association studies (GWAS) have extensively been used to identify agriculturally important genes. However, complex traits such as elemental accumulation are often associated with potentially hundreds of statistically significant SNPs, many of which are located outside known gene models. Identifying causal genes for complex traits quickly become unwieldy even using straightforward marker to gene mapping such as the closest flanking gene.
Here, we present a computational framework called Camoco (Co-analysis of molecular components) that integrates loci identified by GWAS with co-expression networks to identify a focused set of candidate loci with functional coherence. This framework analyses the overlap between candidate loci generated from GWAS and the co-expression interactions that occur between them. Camoco implements a suite of functions that perform SNP-to-gene mapping, builds and functionally validates co-expression networks, and provides a robust bootstrapping model to evaluate the statistical significance of candidate loci. Maize co-expression networks were constructed to represent three different biological contexts: whole plant, genotypic diversity; tissue and developmental variation; and genotypically diverse maize root tissue. Overlap between networks and GWAS was simulated using GO terms in order to establish the expected 'discoverable landscape' of integrating these diverse functional data types. These results quantify the uncertainty in SNP to gene mapping that results from the decay of linkage disequilibrium over many Kb.
Using Camoco to evaluate an empirical dataset, candidate genes were identified from GWAS SNPs associated with elemental accumulation in maize kernels. On average, candidate gene lists identified by GWAS were reduced by two orders of magnitude by integrating co-expression network information, which produced a focused set of candidates with both strong associations with the phenotype as well as evidence for functional coherence in the co-expression network. Camoco is freely available at **http://github.com/schae234/Camoco**.

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**P19**

## CGAT a CRISPR sgRNA Design Tool

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Targeted genome editing is now possible in nearly any organism and is widely acknowledged as a biotech game-changer. Among available gene editing techniques, the CRISPR-Cas9 system is the current favorite because it has been shown to work in many species, does not necessarily result in the addition of foreign DNA at the target site, and follows a set of simple design rules for target selection. Use of the CRISPR-Cas9 system is facilitated by the availability of an array of CRISPR design tools that vary in design specifications and parameter choices, available genomes, graphical visualization, and downstream analysis functionality. To help researchers choose a tool that best suits their specific research needs, we review the functionality of various CRISPR design tools including our own, the CRISPR Genome Analysis Tool (CGAT; http://cropbioengineering.iastate.edu/cgat).

**P20**

## Comparative analysis of C4 photosynthesis genes in two independent origins of C4 in grasses

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C4 photosynthesis evolved as a response to a drop in atmospheric carbon dioxide levels. Despite the complexity of the biochemical and anatomical changes required to convert a plant using the C3 photosynthetic cycle to one using the C4 photosynthetic cycle, C4 photosynthesis evolved independently in estimated 40 times, including 22-24 independent origins among the grasses. However, so far, only two of these origins are represented by species with sequenced genomes. If there are differences in the ways different lineages developed their C4 photosynthetic cycles, a better understanding of how C4 photosynthesis evolved could provide insights on how to improve photosynthetic efficiency in maize. This pilot study focused on the ribulose bisphosphate carboxylase small chain 1A gene (RBCS-1A) The C4 species represent two independent origins of photosynthesis, one comprises maize and sorghum and the other is represented by setaria. On the setaria C4 origin we find high total RBCS-1A expression involved both tandem and unlinked duplications of the RBCS-1A gene, while in maize and sorghum there probably was either a duplication followed by deletion of the ancestral gene copy conserved at a syntenic location in the genomes of other grass species or a direct translocation. As a future work we will have access to 12 genomes from grass species including 10 panicoid species (*Zea mays, Andropogon virginicus, Sorghum bicolor, Paspalum vaginatum, Setaria italica, Urochloa fusca, Panicum virgatum, Panicum hallii, Dichanthelium oligosanthes, Chasmanthium laxum*) representing 3 independent origins of C4 photosynthesis and including the first two C3 panicoid species with sequenced genomes. The analyses will be expanded to span many existing core photosynthesis and C4 photosynthesis related genes to understand differences in how different C4 grass lineages evolved.

Funding acknowledgement: Department of Energy (DOE), National Counsel of Technological and Scientific Development (CNPq), LASPAU Academic and Professional Programs for the Americas

**P21**

## Conservation and divergence of synthetic gene regulation in response to stress in maize and relatives

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Among panicoid grasses, there are cold sensitive species (like maize and sorghum) as well as cold tolerant species. Syntenic genes in these species may respond differently to cold according as a result of either cis-regulatory variation in promoters or changes in upstream trans-regulators. We generated data on cold stress induced changes in gene regulation in five panicoid grass species: maize, sorghum (*Sorghum bicolor*), and foxtail millet (*Setaria italica*) and two wild species: *Urochloa fusca* and *Paspalum vaginatum*. The majority of differentially expressed syntenic genes responded in only a single species. However, upstream transcriptional regulators as well as genes with larger islands of conserved sequenced in their promoters showed greater conservation of expression pattern in response to cold. A more detailed time-course experiment across three species is contributing to the development of more effective statistical methods for scoring the conservation or divergences of gene regulation between syntenic orthologs in closely related species. Future work will incorporate data on lipid metabolism collected in parallel with our RNA-seq data as well as information on changes in open chromatin both across species and within the same species in response to stress. Ultimately this work will lead to a better understanding of how plant stress response networks evolve and change over time as well as the specific changes involved in creating cold tolerance, an adaptive advantage in temperate climates.

Funding acknowledgement: UNL Start-up Funds

**P22**

## Conserved and lineage-specific alternative splicing of orthologous genes in maize, sorghum, and setaria

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Plant genes are frequently observed to produce multiple, alternatively-spliced transcripts, however for many genes the functional role of alternative transcripts remains uncharacterized. In most cases, functional genomic features will exhibit greater conservation across related species than do selectively neutral genomic features. Here we studied the evolutionary conservation of specific alternative splicing events using a set of conserved syntenic orthologous genes from maize, sorghum (Sorghum bicolor), and foxtail millet (Setaria italica) and RNA-seq datasets collected as triplets from the same tissues grown under the same conditions in all three species. In each species approximately 20% of expressed genes showed evidence of two or more expressed splice isoforms. A set of 1269 sorghum genes with exactly two observed splice isoforms and exactly one conserved syntenic ortholog each in maize and setaria were selected to study the conservation of alternative splicing across species. Among this set of genes, 417 (15.9%) 457 (17.5%) and 193 (7.37%) showed evidence of two or more expressed splice isoforms in maize, setaria, or both, respectively. However, in only 1/3 of the cases where the presence of splice isoforms were conserved were the two sorghum splice isoforms were represented in both setaria and maize. These same data are now allowing us to characterize conservation and divergence in isoform abundance ratios across species, as well as interspecific conservation of isoform-specific expression regulation.

Funding acknowledgement: China Scholarship Council

**P23**

## Conserved gene targets between the maize RGH3 and human ZRSR2 RNA splicing factors

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Most eukaryotic transcripts contain introns that are removed by spliceosomes. Eukaryotic lineages often have two different spliceosomes. The major spliceosome removes the common U2-type introns, and the minor spliceosome removes rare U12-type introns. The *rough endosperm3* (*rgh3*) locus encodes the maize ortholog of the human RNA splicing factor, ZRSR2. *ZRSR2* mutations are associated with myeloid dysplastic syndrome (MDS), are drivers toward cancer, and cause mis-splicing of U12-type introns. Analogous to MDS, maize *rgh3* mutants show aberrant endosperm cell differentiation and excess proliferation in tissue culture. RNA-seq analysis of *rgh3* found that 71% of U12-type introns are mis-spliced in *rgh3* with adjacent U2-type introns affected in some transcripts. RGH3 co-localizes with the U2 Auxiliary Factor (U2AF) subunits and may interact with U2AF based on bimolecular florescence complementation. These results suggest that U2 and U12 spliceosomes may function cooperatively to identify splice sites. The similarity of defects in human and maize mutants indicates conserved roles for U12 splicing in cell differentiation. Genes mis-spliced in both *rgh3* and *ZRSR2* mutants identify cell cycle and protein glycosylation as common pathways disrupted. Based on reciprocal best match homologs, 42 maize genes have significant retention of a U12-type intron in *rgh3* and correspond to 16 human genes that also show splicing defects in *ZRSR2* mutants. Protein alignments identified U12-type intron positions for the maize/human homologous gene families. Divergent positions of U12-type introns in E2F3 may explain differences in the cell proliferation phenotypes of MDS patients and *rgh3* mutants. Seven gene families have identical U12-type intron positions in human and maize. Based on this deep conservation of intron position and cellular phenotype, we propose a model in which the minor spliceosome pathway for cell differentiation existed in eukaryotes prior to the divergence of plants and animals and their independent evolution of multicellular development.

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

**P24**

## Differential Expression of Maize Memory Genes and Phenotypic Change in Response to Priming and Cold Stress

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Acclimating plants to abiotic conditions is a phenomenon that can be observed in nature throughout changing seasons as well as within lab settings. Plants can adjust to abiotic stresses through biochemical changes controlled by transcription of genes. Trained plants can be produced by pre-exposing them to a lesser stress, allowing them to recover, then exposing them to a greater, longer stress. It is hypothesized that trained plants will tolerate the second harder stress more than untrained plants. In this experiment, B73 Maize seedlings were trained to cold stress while their growth, and greenness were measured to determine plant health. Four treatment groups with over sixty replicates were tested; Primed and Stressed, Primed, Stressed, and Control. Half of these plant's growth was measured daily as total height, and finally sacrificed at the end of the cold stress protocol to obtain a percentage representing green area of the 3rd leaf through image analysis and a chlorophyll concentration per gram through methanol extraction and absorbance calculations. The other half was sacrificed after 2 hours of final stress for RNA extraction to be RNA sequenced. The results showed that priming offered no benefits to the maize seedlings immediately after stress. Primed and Stressed plants responded very similarly to Stressed plants both phenotypically and transcriptomically. However, when allowed to grow and recover after the final stress, Primed and Stressed plants recovered faster than Stressed plants indicating that priming may have a more beneficial effect during stress recovery than stress tolerance. In the future, phenotypic and transcriptomic analysis should be conducted during the stress recovery period.

Funding acknowledgement: National Science Foundation (NSF)

**P25**

## Dissecting the metabolic network for silk extracellular cuticular lipids in maize: Contrasting linear regression models and correlation networks during silk development and different genotypes

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Extracellular cuticular lipids (ECLs) are prevalent on maize silks, comprising ~2% of silk dry weight. These cuticular lipids provide a hydrophobic barrier between silks and the external environment and provide protection against stresses. ECLs are comprised of saturated and unsaturated hydrocarbons, fatty acids, and aldehydes of varied carbon chain lengths (16-35 carbons). Lipid constituents within the silk surface metabolome are biochemically linked via enzymatic reactions. This metabolic reaction network includes a series of reactions that elongate fatty acids by the iterative additions of 2-carbon units, and subsequent reactions that can convert each fatty acid to aldehydes, and saturated or unsaturated hydrocarbons. Accordingly, we have proposed a metabolic reaction network that incorporates precursors, intermediates and end-point metabolites at each observed carbon chain length. To assess how the proposed network is impacted by silk development and genetics, we have profiled ECLs from silks at different stage of development from inbreds B73 and Mo17, and their reciprocal hybrids. Correlation analyses and regression modeling were then applied to the resulting metabolome. For each proposed biochemical reaction, a regression model was developed based on a series of precursor-product ratios between the abundance of hypothetical reactants (e.g. fatty acids) and products (e.g. aldehydes) at individual chain lengths. Such models permit mathematical interrogation of the proposed network topologies during silk development by different genotypes. Concordant results of regression and correlation analyses have produced several salient findings. One of these is that during silk development the metabolic network is more divergent across lipid chain lengths when Mo17 alleles are present (i.e., the network in B73 is simpler than in Mo17, and Mo17xB73 and B73xMo17 hybrids). This and other revelations will be discussed in the context of our teams' systems biology approach to understand the metabolic and genetic networks for ECL production as affected by development and environmental modifiers.

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

**P26**

# DNA replication dynamics in maize root tips

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During growth and development, all plants and animals must replicate their DNA, using a regulated process that ensures all sequences are completely and accurately replicated. The time at which particular sequences replicate during S phase has been studied extensively in yeast and animal systems, but very little is known about replication timing programs in plants, with the exception of one lower resolution study in Arabidopsis. We set out to understand the replication timing program of the maize B73 genome, and relate it to transcriptional activity, epigenetic state, and genome architecture. Root tips were pulse-labeled with 5-Ethynyl-2´-deoxyuridine (EdU), and nuclei flow sorted based on EdU incorporation and DNA content into early, mid and late stages of S phase. Labeled DNA was immunoprecipitated from each stage, and Illumina sequenced to generate whole-genome replication timing profiles. Data are presented as the ratio of filtered, uniquely mapped reads from newly replicated DNA to reads from non-replicating G1 DNA. On inspection, we discovered that most places in the genome show some replication activity at two or more times during S. This observation led us to develop a computational approach that automatically optimizes parameters specific to the dataset, and identifies the predominant replication time of any given locus. Using this approach, we found that 66% of the genome can be classified as predominantly replicating at a single time during either early (22%), mid (24%), or late (20%) S phase. The rest of the genome was classified as predominantly replicating at two or more times, including early and mid (18%), mid and late (14%), early and late (0.2%) or pan-S (0.3%). Further observations on the general positioning of early, mid and late replicating DNA on maize chromosomes will be presented. We also relate replication timing to whole genome data for transcription, several histone modifications, and DNA methylation.

Funding acknowledgement: National Science Foundation (NSF)

**P27**

# DNS - NUPRIME: Differential Nuclease Sensitivity for Nuclease Profiling as an Integrative Resource for Maize Epigenomics.

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Eukaryotic chromosomes consist of DNA-protein complexes referred to as chromatin. The current understanding of the relationship between chromatin structure and genome behavior is still relatively underdeveloped. The goal of this project is to identify and map genome-wide changes in chromatin structure in a core set of diverse maize tissues: stem cells from root and shoot tips, immature ear, developing nutritive seed tissue (endosperm), and pollen sperm nuclei. Our assay for genome-wide nuclease profiling using micrococcal nuclease (MNase) measures differential nuclease sensitivity (DNS-seq) across the entire maize genome. This results in a genomic annotation and quantitative mapping of MNase-sensitive footprints known to colocalize with open chromatin in general, and with evolutionarily conserved noncoding sequences, transcription factor binding sites, and active promoters in particular. This method uniquely maps specialized and functionally important regions of the genome while greatly enhancing the information content of nucleosome position data, providing new opportunities to connect epigenomic data across disparate projects. NUPRIME chromatin profiling workshops at FSU (one held in August of 2015 and one planned for May of 2016) provide hands-on training - from tissue to browser - for participants wanting to apply DNS-seq to their own experimental system. We also show how conventional nucleosome occupancy mapping was used to uncover drought-induced, MNase-resistant footprints in a select set of ~400 genes using microarrays. In summary, DNS-seq can be described as a highly informative genomic assay that detects chromatin structural dynamics coupled to genetic functions and resolved on a very small scale – often one nucleosome (~150 bp) or smaller. As experimental data, these profiles accelerate discovery while bridging the growing layers of epigenomic information associated with chromatin structure and genome response in maize.

*Dr. Hank Bass and Dr. Daniel Vera will be co-presenting this poster.

Funding acknowledgement: National Science Foundation (NSF)

**P28**

# Evolution of maize during post-domestication expansion across the Americas

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Maize, like many crop species, colonized a much larger area following domestication in a narrow center of origin. Many population genomic questions regarding the demography of maize during this expansion remain unanswered. For example, how did the effective population size of maize change during its spread? How has range expansion affected genetic diversity and the accumulation of genetic load? To what extent have maize landraces been subject to introgression from closely related teosinte during expansion into sympatry with these taxa? Here, we report high-depth (20-40X) re-sequencing of 31 maize landrace genomes spanning the Americas. Genome-wide demographic analyses reveal a continuous genetic bottleneck in maize landraces starting from approximately 10,000 BP and lasting to 1,000 BP in all surveyed regions, with a substantially stronger bottleneck occurring in the Andes of South America. These results suggest a history of serial founder effects in maize during expansion, a finding we have further validated using publicly available genotyping-by-sequencing data from 3520 landraces. Maize, in this respect, is similar to humans, and other recently expanding species. As predicted by range expansion theory, the Andean landraces, located at the expansion wave front of maize, demonstrate the lowest genetic diversity, the highest level of derived homozygous genotypes and runs of homozygosity, and the highest genetic load. Previously detected introgression from teosinte (Zea mays ssp. mexicana) into Mexican highland landraces was confirmed using our genomic data and unreported introgression from this same taxon was found extending into the Guatemalan highlands and the southwestern US. In addition to clarifying the demography of maize during its history of spread, our work has practical implications for maize breeding. For example, efforts to improve Andean material may be particularly challenged by its high level of genetic load, a symptom of its evolutionary history.

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

**P29**

# Evolution of temperate maize in North America

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Four thousand years ago, maize began migrating from Mexico to the geographically diverse Southwestern US. Over 2,000 years of population selection, early Puebloan people adapted this germplasm to the short-season uplands. We show that the resulting germplasm became the Northern Flints, which provide temperate adaptation to modern US, Chinese, and European varieties. We combined mapping and genomic prediction trained on large modern inbred populations with population analyses from modern landraces and ancient DNA from the Southwest to understand the biological basis, timing, and population dynamics underlying early flowering. We addressed the biological basis of early flowering using six modern populations, totaling nearly 15,000 lines. We remapped flowering on 81 million projected whole-genome sites using published SNP-based and novel variance component approaches, and combined results across populations in a meta-analysis. We identified significant regions resulting from the Southwest adaptation by comparing results to Fst calculated between Northern Flint and tropical lines. Enrichment in functional, evolutionary conservation and population genetic annotations underlying significant regions elucidates the biology underlying flowering time, clarifying the mechanisms of adaptation. We investigated the population dynamics underlying early flowering with GBS-genotyped and partially phenotyped modern SW-focused landraces and 14 whole-genome sequenced cobs from the Turkey Pen archaeological site, an early intensifying-agricultural site in the uplands. Population analyses of Turkey Pen and modern landraces suggest that Turkey Pen is ancestral to both the Northern Flints and modern Southwestern landraces. We validated the flowering model trained on modern inbreds with our phenotyped landrace population, demonstrating good prediction accuracy, and applied it to Turkey Pen to estimate extent of adaptation. We also present segregation results for known early flowering loci in Turkey Pen, such as the MITE insertion at vgt1. These data suggest that early flowering was mostly in place by 2,000 years ago, and relied on standing variation from lowland north Mexico.

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

**P30**

## Finding Groups of Phenotypes under Combined Stresses by Clustering

(submitted by Avimanyou Vatsa <akvhxd@mail.missouri.edu>)
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Crop improvement must accelerate with increasing human population and environmental changes. We analyzed data from 90 different inbred lines subjected to combined drought and nitrogen deprivation in the greenhouse. Three output variables were measured before and after treatment: the difference in height (Δ h); the difference in canopy width (Δ c); and the difference in stem diameter(Δ s). We used a clustering procedure to identify the distinct phenotypes exhibited by the lines.
To understand the genetic correlation/pleiotropy of multiple phenotype we used clustering. Since each line has a different basal growth pattern and we wanted to compare among the lines, we first rescaled and standardized the data. We then used the nonparametric SAS procedure MODECLUS to cluster the data. We tuned **MODECLUS's** parameters threshold, radius, unclassified points and corresponding number of clusters to optimize the number and stability of the clusters. The tuning of the parameter give the exact number of clusters with zero number of unclassified points; and the clusters predict groups of similar phenotypes.

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

**P31**

## Fine scale nucleosome positioning coupled with centromeric chromatin in maize

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The nucleosome consisting of ~147 bp of DNA wrapped around on octamer of histones is the fundamental unit of chromatin in eukaryotes. Centromeres mediate kinetochore assembly for the attachment of spindle microtubules during mitosis and meiosis. CENH3, the centromere-specific histone H3 variant, is one of the most characterized epigenetic elements involved in centromeric chromatin. Determination the CENH3 nucleosome positioning in the centromeric region is the key to an understanding of centromere chromatin and function. Here we report the ChIP-seq analysis with CENH3 antibody, which reveals a comprehensive view of the centromeric nucleosome landscape in maize native and de novo centromeres. CENH3 nucleosomes in two maize well-assembled centromeres, 2 and 5, are mainly positioned on the repetitive CentC and CRM sequences. We identify ~2000 and ~3000 high-confident CENH3 nucleosomes in the 3.4-Mb-Cen2 and 4.8-Mb-Cen5, which indicates CENH3 nucleosomes occupy only a small proportion of centromere. Three neocentromeres Dp3a, Derivative 3-3 and sDic15 also reveal fine structure of CENH3 nucleosome positioning. The dynamic change of DNA methylation level before and after neocentromeres formation in neocentromere is not parallel with CENH3 nucleosomes in the centromeric region. Our results indicate centromeric histone compositions are dynamically regulated with respect to different factors, such as DNA sequences and methylation.

Funding acknowledgement: National Natural Science Foundation of China

**P32**

## Gametophytic Cross Incompatibility in Maize: Sequencing the *Ga1-m* Locus

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The inability of certain genotypes to successfully pollinate other genotypes is attributed to unique components referred to as gametophyte factors. Both male and female functions have been described. The female function is found in silks of select genotypes and acts as a barrier preventing pollen tube growth of certain pollen genotypes. The male function refers to a component found in pollen of select genotypes that allows the pollen to overcome the silk barrier. This research is focused on the gametophyte factor *Ga1*. Three alleles at the *Ga1* locus have been described: *ga1*, *Ga1-s* and *Ga1-m*. *ga1* is the most common genotype in maize production fields and it lacks a male and female function. *Ga1-s* genotypes possess a male and female function. *Ga1-m* genotypes only possess a male function. Previously completed research by other groups have successfully fine mapped the *Ga1* locus on the short arm of chromosome 4, however the causative gene or polymorphism has yet to be identified. The *Ga1-m* region was backcrossed into a W22 inbred line by Dr. Kermicle. A BAC library was constructed by Dr. Evans and four BACs with a predicted size of 100 kb each were identified and sequenced using single end Illumina MiSeq technology. The resulting 12,840,834 reads from the BACs were subjected to de novo assembly using MIRA 4.0.2. Contigs resulting from de novo assembly were subjected to contig joining using GAP 5. Post assembly and contig joining resulted a final sequence with 15 contigs and a total consensus length of 398,391 bp. Assembled contigs were subjected to gene prediction using Softberry, FgenesH. A total of 59 predicted genes were found within the contigs. Through sequencing the *Ga1-m* locus and running comparisons between the sequences of *ga1* and *Ga1-s* genotypes we have been able to identify candidate genes for the three *Ga1* phenotypes.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P33**

## GeneWordSearch: An open source tool for keyword enrichment analysis of gene sets

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Through the use of modern sequencing technologies, large scale analyses in agricultural species have uncovered relationships among sets of genes that are largely uncharacterized. Traditional functional analyses, such as Gene Set Enrichment Analysis (GSEA), relies on high quality annotations, which are typically derived from literature curated ontologies. However in maize, these knowledge bases can be difficult to interpret due to a lack of species specific understanding of genes and a heavy reliance on functional annotations mapped from orthologs in model species. Here, we introduce a simple tool called GeneWordSearch (GWS) that uses text mining to discover gene-word associations using simple text-based gene annotations combined with putative annotations of homologous genes in other species. This differs from traditional GO Term enrichment or GSEA analysis in that text associated with each gene is processed individually outside of the context of an ontology term. Statistical analysis is performed by calculating the enrichment of the words themselves, resulting in a list of the most statistically over-represented words for a given query set. GWS is publicly available via a simple web interface and can be accessed at http://csbio.cs.umn.edu/gws. This web interface provides a pre-built dataset created with annotations derived from several publicly available datasets, but also allows users to upload their own custom annotation datasets as simple text files. Additionally, GWS provides a general use API that allows easy integration into existing projects. We also integrated GWS into an existing web based tool, the Coexpression Browser (COB). The integration of these two tools enables not only the discovery of putative functional sub-networks, but also, for networks of unknown function, a starting hypothesis suggesting functions represented in the network using GWS. GWS is available as an open source python module from either PyPI or GitHub.

Funding acknowledgement: National Science Foundation (NSF)

**P34**

## Genome-Guided Transcriptome Phylogenomics of the Paniceae Grasses

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The tribe Paniceae is a relatively young (10-20MYA) group of grasses that includes economically important forage grasses, millets, and noxious weeds. It also contains many different lineages of $C_4$ photosyntheis, including species representing each of the three classically defined $C_4$ subtypes. Although the relationships among members of the Paniceae have been studied in detail for many years, parts of the tribe's phylogenetic history remain elusive; perhaps in part due to its young age and rapid radiation. To better understand the evolutionary history of the tribe we developed a method we call genome-guided transcriptome phylogentics which uses sequenced genomes from within and without of the tribe as anchor points for de novo assembled transcriptomes. This allowed us to determine the phylogenetic histories of over 2,000 genes from ~40 Paniceae taxa. Examination of these gene trees revealed the most likely nuclear phylogeny of the group as well as several instances of gene tree species tree incongruence within the Paniceae.

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**P35**

## Genome-wide analysis of the lysine biosynthesis pathway network during maize seed development

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Lysine is one of the most limiting essential amino acids for humans and livestock. The nutritional value of maize (*Zea mays* L.), one of the most important cereal grains, is reduced by its poor lysine content. To better understand the lysine biosynthesis pathway in maize seed, we conducted a genome-wide analysis of the genes involved in lysine biosynthesis. We identified two genes encoded the core enzyme dihydrodipicolinate synthase (DHDPS), and determined that they played different roles in lysine biosynthesis during maize seed development. A coexpression network of lysine biosynthesis pathway genes (LBPGs) was constructed. Forty-four TF families containing 217 transcription factors were identified. Phylogenetic analysis of the LBPGs from different plant species revealed different phylogenetic relationships. Additionally, several expression quantitative trait loci (eQTLs) of LBPGs were identified. Our results should be helpful for the elucidation of the network of lysine biosynthesis pathway in maize seed and for the improvement of maize seed lysine content.

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**P36**

## Genome-wide analysis of variation in gene-copy and paralog number in the HapMap2 collection of maize and teosinte inbreds

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Differences in gene-copy and paralog number are thought to contribute significantly to genetic variation among maize inbreds as well as potentially differentiating maize from ancestral teosinte. However, much remains unknown about the extent of variation in gene-copy and paralog number in maize and the processes of gene creation and gene loss that underlie this variation. Thus far, genome-wide analysis of gene-copy and paralog number has been limited to a small set of inbreds that have assembled genome sequences. However, un-assembled, low-coverage WGS datasets are available for a much larger set of inbreds and accessions included in the HapMap2 collection. We reasoned that low-coverage WGS data was sufficient to estimate relative frequencies of short-oligomer sequences (kmer's) derived from a reference gene (e.g. B73). To test this approach we first analyzed variation in ZmCCD1 copy number at the White cap 1 (Wc1) locus in the HapMap2 dataset. The resulting short-kmer based estimate of ZmCCD1 gene copies agreed well with qPCR-based estimates. Moreover, within the set of inbreds used as NAM parents we found a remarkably strong correlation (r^2=0.94) between ZmCCD1 copy number and gene expression data obtained from QTELLER.ORG. We have thus extended this kmer approach to a genome-wide level using the 39,450 genes in the filtered-gene set from B73 as a reference. The resulting genome-wide and population-wide profiles illuminate haplotype relationships within maize and reveal a novel set of candidate domestication genes that are enriched in maize relative to teosinte. By utilizing existing low-coverage and variable-quality WGS data, kmer analysis provides a fast, robust method for comparing structural variation between genomes such as maize and teosinte.

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**P37**

## Genome-wide characterization of maize promoter regions and transposable element composition

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The association between repeat elements and gene regulation has been largely suspected. The large and complex maize genome provides an ideal opportunity to study the contribution of repeats, particularly of transposons (TEs), to the architecture of regulatory regions. Several examples of gene regulation related to TEs have been identified in the maize genome. However, scarcity of methods that allow large-scale experimental identification of promoter regions makes it difficult to study presence of TEs in gene regulatory regions.
Recently, we applied CAGE (cap analysis of gene expression) to identify maize transcription start sites (TSSs) in two tissues (Root and Shoot) from two inbred lines (B73 and Mo17). Using TSSs locations and their expression levels, we characterized core promoter regions associated with sharp and broad transcriptional initiation. We discovered that most of maize promoters are sharp, an observation that differs from metazoan model organisms. Moreover, this dataset revealed an unanticipated flexibility in the usage of core promoters between tissues (root and shoot) and inbred lines, which was evident in ~1,500 genes that differed significantly in TSS usage.
Taking advantage of the CAGE information, we investigated the presence and preferences of TEs flanking the TSSs. Our analyses showed that certain TE families are enriched in flanking TSS regions, when compared to their overall genomic abundance. Moreover, we observed that the proximity of a TE to the TSSs is different for transcription units related to coding protein genes than for transcription units located in the intergenic regions (related to what we consider putative enhancers).
Our findings provide a genome-wide atlas of TEs related to transcription initiation. The TSSs-TE atlas therefore warrants further exploration to improve our understanding of the role of TEs in the architectural organization of regulatory regions.

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**P38**

## Genome-wide mapping of hypersensitive footprints in chromatin of developing maize tassels predicts regulators of inflorescence architecture

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Genome-wide mapping of nucleosome position and open chromatin during early stages of maize inflorescence development can predict active regions of transcriptional activity regulating developmental transitions. Open chromatin signatures inform the location of cis-regulatory elements where transcription factors (TFs) and other regulatory proteins interact to control gene expression. To map signatures of open chromatin and nucleosome occupancy, we performed micrococcal nuclease (MNase)-mediated digestion of chromatin using intact nuclei extracted from B73 maize tassel primordia (two biological replicates; pools of ~200 2-5mm tassels). We used two different concentrations of MNase to obtain differential nuclease sensitivity (DNS) profiles, where light digests produce MNase-sensitive footprints and heavy digests produce MNase-resistant footprints. Paired-end sequencing of MNase-seq libraries generated millions of reads per replicate, which were mapped to the maize genome to identify thousands of hypersensitive sites (light:heavy signatures). The distribution of hypersensitive sites indicated that the vast majority them are enriched in promoter and intergenic regions. We also integrated transcriptome profiles and TF binding maps from B73 tassel primordia to determine whether hypersensitive sites associated with actively transcribed genes and/or were bound by developmental TFs. Preliminary analyses showed strong associations with binding of KNOTTED1 and FASCIATED EAR4 TFs, regulators of meristem maintenance and size, respectively, and with inflorescence-specific RAMOSA1, suggesting that these open chromatin signatures can predict TF binding in maize inflorescence development. Moving forward, our goal is to identify regulatory regions associated with developmental genes, predict and validate trans-acting factors that bind them. We are leveraging the grass model system Setaria viridis to disrupt predicted regulatory regions using CRISPR/Cas9-based genome editing. As a test case, we have generated a liguleless1 (lg1) mutant in Setaria using CRISPR/Cas9 and confirmed a comparable leaf angle phenotype to maize. We are now using this system to test predicted regulatory regions proximal to the lg1 gene in maize.

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**P39**

## High-latitude adaptation through early floral induction in maize

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The progressive adaptation of maize germplasm to northern latitudes required the selection of early flowering genotypes with tolerance to low temperatures. Flint is a major germplasm pool in maize, well adapted to colder climates, which represents an ideal model to gain insights into the molecular basis underlying modulation of flowering time. We performed whole-genome screens for signatures of selection specific to 66 temperate Flint inbred lines compared to 70 temperate Dent lines based on high-density genotyping data. Focussing on the flowering network, the majority of Flint candidate genes were associated with signal integration and flower development. In contrast to this finding, the Dent candidate genes were primarily involved in light perception and photoperiod dependent pathways. We demonstrate the functional relevance of the Flint candidates for promoting flowering in a Dent-Flint introgression library. Whole-genome sequence data revealed that selection acted preferentially on the regulatory regions of the genes. Exploring the genetic variation of the candidate genes in more than 900 individuals derived from 38 European landraces corroborated the impact of the identified candidates on the adaptation of Flint to higher latitudes in temperate European climate zones.

In summary, our results show that photoperiodism independent floral induction enabled the progressive adaptation of temperate maize to cooler and shorter vegetation periods. The Flint-specific haplotypes of the identified candidates constitute a promising source for the short-term adaptation of other maize germplasm pools to higher latitudes, which is of particular relevance in the context of ongoing global warming and its negative effect on maize yield.

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**P40**

## Identification of genetic variants modulating alternative splicing in maize kernel

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Alternative splicing enhances transcriptome diversity and plays important roles in regulating plant processes. By transforming multivariate splicing ratios into univariate geometrical distances and combing with mixed linear model, we report a genome-wide association study (GWAS) to identify splicing quantitative trait loci (sQTLs) in developing maize kernels from 368 inbred lines. We detected 3,790 unique sQTLs involving 2,700 genes, with cis-sQTLs predominating. Small changes in relative isoform usage dominate most of sQTLs. sQTLs appear to be under relatively independent genetic control from overall mRNA level, in which cis-sQTLs are particularly enriched in splice-site regions. We identified 55 putative trans-acting splicing regulators, several of which are splicing-related proteins. Among them, *ZmGRP1*, an hnRNP-like glycine-rich RNA-binding protein, regulates the largest trans-cluster. We determined that 112 sQTLs colocalize with previous GWAS signals for downstream phenotypes, most of which occurred without changes in overall gene expression, underscoring the importance of alternative splicing in phenotypic variation.

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**P41**

# Investigating diversity and possible functions of G-quadruplexes in regulatory regions of maize genes

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G4-quadruplexes are reversible DNA structures that likely function in gene regulation, but exactly how they work is not known. G4 DNA can be predicted from sequence motifs such as the pattern G-G-G-N(1,7)-G-G-G-N(1,7)-G-G-G-N(1,7)-G-G-G-N(1,7). In the maize genome, G4 motifs were found to occupy non-random sites including antisense 5' UTR hot spots in genes associated with low energy signaling and responses, including hypoxia, low sugar, and nutrient deprivation1. This enrichment suggests that maize G4 DNA may play a role in energy stress response. We are conducting analyses that seek to determine whether and how genes harboring G4 elements: (1) contribute to plant developmental processes and stress response, (2) vary in constitution across diverse germplasm, and (3) could be combined (through plant breeding or gene editing) to modulate the stress response. Here we describe our initial results and outline next steps for these investigations

**P42**

# KBCommons: A multi 'OMICS' integrative framework for database and informatics tools

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Advancement of next generation sequencing and high-throughput technologies has resulted in generation of multi-level of 'OMICS' data for many organisms. However, these data are often individually scattered across different repositories based on data type, making it difficult to integrate them. We have addressed this issue through our in-house developed Soybean Knowledge Base (SoyKB) framework, a comprehensive web-based resource that bridges translational genomics and molecular breeding research in soybean. It acts as a centralized repository for soybean multi-omics data, and is equipped with an array of bioinformatics analytical and graphical visualization tools. It is available at http://soykb.org and has proven to be a great success with more than 400 registered users.

Users working on other biological organisms including plants, animals and biomedical diseases have similar needs and the developed framework can be easily expanded to make the visualization and analysis tools function for other organisms, without having to reinvent the wheel. To achieve this we have developed KBCommons, a platform that automates the process of establishing the database and making the tools for other organisms available via a dedicated web resource. It provides information for six entities including genes/proteins, microRNAs/sRNAs, metabolites, SNP, traits as well as plant introduction or strains/populations. It also incorporate several multi-omics datasets including transcriptomics, proteomics, metabolomics, epigenomics, molecular breeding and other types. We have currently expanded KBCommons framework and tools to Zea mays, and in future plan to add other organisms including *Mus musculus* and *Homo sapiens*.

KBCommons also provides a suite of tools such as the gene/metabolite pathway viewer, Protein Bio-Viewer, 3D protein structure viewer, heatmaps, scatter plots and hierarchical clustering. KBCommons also has suite for differential expression analysis of omics datasets including venn diagrams, volcano plots, function enrichment and gene modules. It will also incorporate access to Pegasus analytics workflows for analysis in future.

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**P43**

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

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Making a genome sequence accessible and useful involves three basic steps: genome assembly, structural annotation, and functional annotation. The quality of data generated at each step influences the accuracy of inferences that can be made, with high-quality analyses produce better datasets resulting in stronger hypotheses for downstream experimentation. Here we report on our efforts to assess existing functional annotations for maize as well as to generate not only a high-quality functional annotation set for B73, but a freely available pipeline that enables others to carry out the same process on other inbred lines and plant genomes. Our pipeline makes use of methods developed for the Critical Assessment of Function Annotation (CAFA) competition (see http://biofunctionprediction.org/ for details) and enables experts to endorse or reject annotations to further improve the quality of available functional annotations. Preliminary results from the pipeline suggest that a combined system based on multiple methods increases both the number of genes that are assigned at least one functional annotation (GO term) and the quality of functional assignments on average (as compared to the existing Gramene/Ensembl pipeline). A downstream component of the pipeline enables review of functional assignment by experts, which promises to improve the confidence-level of evidence codes associated with GO terms assigned by these computational methods.

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**P44**

## MIGD: An Integrated Maize Inflorescence Genomics Database

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Maize produces more grain than any other crop. Separate male and female inflorescences are produced in maize, the pollen-bearing tassel and grain-bearing ear, respectively. The genetic processes that control tassel and ear development are largely analogous and also underlie inflorescence architecture across the grasses, including other cereal crops that help feed the world. Thus, understanding the regulatory mechanisms controlling maize inflorescence architecture is of especially broad relevance for agricultural research. Likewise, understanding how this regulation is perturbed in response to abiotic stress is increasingly important in the face of a changing environment. For example, early season drought stress can negatively impact yield by disrupting or blocking maize ear development. The NSF-funded Maize Inflorescence Project (PGRP award #1238202) is dissecting how the critical regulators of maize development interact with drought stress. A web-based database, http://www.maizeinflorescence.org, has been developed to integrate various genomics datasets produced by this project (e.g., RNA-seq and ChIP-seq data) as well as other publically available data (e.g., maize genome annotation). The integrated computational tools allow researchers to analyze gene expression, regulatory motifs, metabolic pathways, single nucleotide polymorphisms, and other genomic data in a seamless fashion.

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**P45**

## Natural antisense transcripts are significantly involved in regulation of drought stress in maize

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Natural antisense transcripts (NATs) are a prominent and complex class of regulatory RNAs that are subject to regulation in response to stress. Using strand-specific paired-end RNA sequencing, we identified 1,447 sense and antisense transcript (NAT) pairs in two maize inbreds with different sensitivity to drought, as well as in two derivative recombination lines. We found that one or both transcripts were expressed for 83.28% NAT pairs under water stress (WS) and for 65.65% under well water (WW), with NAT transcription during drought considerably more variable than average. Surprisingly, sense and antisense transcripts in a majority of NAT pairs are significantly correlated and is even stronger under WS. However, NATs were also expressed in a more specific manner than sense transcripts based on Shannon entropy estimates, and with greater difference in expression between the two parental lines. Small RNAs significantly accumulated around NAT pairs, with 21 nt smRNA especially enriched in overlapping regions of sense and antisense transcripts via an undefined mechanism that was greatly effected by Leafbladeless 1, necessary for the biogenesis of trans-acting short-interfering RNAs. Further, NAT pairs are significantly hypomethylated, as measured by MeDIP- and bisulfite sequencing, and are sparsely covered by transposable elements. Antisense gene regions also exhibit higher levels of H3K36me3, H3K9ac, and H3K4me3, but lower levels of H3K27me3, indicating active transcription. Finally, NAT pairs in 386 diverse maize inbreds and 19 segregating populations were specifically enriched for polymorphisms associated with drought resistance. Taken together, the data highlight the impact of epigenetic regulation on NAT expression, and the significance of NATs in response to WS.

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**P46**

## Pan-genome analysis reveals functional variants in maize

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Maize genome is highly complex in terms of its genomic structure. Particularly for the maize pan-genome, including multiple de novo maize genome assemblies (B73, CML247, W22, PH207, Mo17), it becomes intractable to conduct effective analysis due to the high amounts of structural variations. To reduce the complexity, we performed a novel kmer analysis using multiple maize assemblies to score uniqueness (uniqueness score (UScore)) of every single base of maize genome. The results showed that about 15% genome are unique in maize. Known functional elements, such as CDS and MNase hypersensitive sites, are in these unique regions. However, functions of two thirds of unique genome remain unknown. In addition, we performed variance partitioning analysis on 45 traits using the nested association mapping (NAM) populations. As expected, the unique regions of genome explain a large amount of heritability. However, we found genomic regions which are duplicated for a few times generally showed much more enrichment (up to 40 folds) in terms of explaining heritability. We hypothesized that these moderately duplicated regions are enriched for regulatory elements of genes and/or they pick up large effect of variants in highly conserved genome by LD.

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**P47**

## Phylogenetic and transcriptome analysis of CBF and ICE gene families in maize

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Along with other mechanisms, plants utilize transcriptional responses to tolerate abiotic stress. The CBF (cold binding factor; also known as DREB) family of transcription factors has been implicated in abiotic stress response in a number of plant species and likely play important roles in maize as well. The more recently discovered ICE (inducer of CBF expression) transcription factor family likely regulates CBF expression to control cold inducible genes. We were interested in identifying members of the CBF and ICE transcription factor families that are induced in response to cold or heat stress in maize. A phylogenetic analysis of CBF and ICE genes revealed sub-groups that identified maize orthologs of genes that exhibited transcriptional roles in cold-responses in rice or other monocots. Three RNAseq datasets profiling transcriptional response to cold stress over different time courses and in distinct inbred genotypes were used to monitor the expression of CBF and ICE genes. These datasets include a cold-stress time course experiment in 11 day Mo17 seedlings, a cold-response priming experiment from 14-24 day old Mo17 seedlings, and a separate cold experiment in 14 day old seedlings of B73, B37, Mo17, Oh43, and PH207 inbreds. A subset of the CBF and ICE genes are strongly induced by cold stress while many others are not affected. In many cases, the specific CBF or ICE genes that respond to cold-stress are related to rice or Arabidopsis CBF/ICE genes with demonstrated roles in cold response. The transcriptome data was also utilized to evaluate natural variation among different inbred lines for CBF and ICE responses to cold stress. This project identifies a set of candidate transcription factors that are expected to play important roles in maize responses to seedling cold stress.

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**P48**

## Polishing the highly repetitive centromere regions of Refgen V4

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Maize centromeres are marked epigenetically by the histone H3 variant cenH3 and composed primarily of the satellite repeat CentC and members of the Centromeric Retrotransposon (CR) family. Correct assembly of centromeres is essential for reconstructing the evolution of these regions. A recent effort to improve the maize reference genome (V4) using Single Molecule Real-Time sequencing technology (PacBio) and NanoChannel Array from BioNano has resulted in the closure of numerous gaps and correct orientation of many sequence fragments genome-wide. However, the inability of this approach to anchor 28 Mb (254 high-quality contigs averaging 100 kb) of sequence results in several large gaps in multiple centromeres, including previously characterized centromeres of chromosomes 2 and 5. Comparison of the 1.8 Mb region containing cenH3 on chromosome 10 in B73 (CEN10), sequenced independently with PacBio technology using a BAC-by-BAC approach, with the corresponding region in RefGen_V4, revealed that a 250-kb region consisting of a segmental duplication and many nested CRs is unanchored in V4. Another region (200-kb) at the downstream edge of CEN10 contains one expressed gene and is correctly assembled, but unanchored in V4. The 8.3 Mb region of chromosome 5 containing the active centromere locations of 28 diverse maize inbreds (CEN5) has eight gaps in V4. Two gaps are due to chimeric contigs that were identified using unique markers (e.g., repeat junctions) and fixed based on Sanger sequence from the corresponding BAC. Unique markers from high-quality, singleton and repeat contigs as well as the raw sequence reads were used to span all but three gaps in CEN5, revealing several additional CR elements. The remaining three sequence gaps are all in CentC regions. Details of the actual number of CR elements present in CEN5 will be provided. We propose to use unique markers to close gaps in the non-CentC regions of all centromeres.

Funding acknowledgement: National Science Foundation (NSF)

**P49**

## RefSeq Curation at NCBI - Adding Value to the Maize Genome Resources

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NCBI created Zea mays RefSeq transcript-protein pairs and the associated NCBI Gene record from the INSDC maize accessions. This is a publicly available resource consisting of a non-redundant collection of sequence records for mRNA and the encoded protein; each pair with an associated NCBI Gene record. Curation of Zea mays RefSeq transcripts creates a more robust and accurate collection of genome resources for the maize research community. Curation involves 1) identifying instances of multiple identical RefSeq transcripts for a particular gene and resolving by merging, 2) identifying instances of genes expressing transcript isoforms and creating the necessary RefSeq accessions,
3) identifying instances of RefSeq transcripts with sequence variations relative to the genome sequence and resolving by creating either a new version of the transcript accession or a new transcript accession, 4) identifying instances of structural and/or sequence deficiencies
in the genome, 5) identifying instances where the annotation endeavors by NCBI and maizesequence.org differentially annotate a gene and 6) identifying instances of the genome sequence lacking a particular gene. Points four and six result in a record in the internal
Genome Problem database at NCBI; the contents of which are shared with MaizeGDB. A consequence of points five and six is that NCBI may be able to still track the missing locus in the NCBI Gene resource and be able to provide a representative RefSeq transcript.

Funding acknowledgement: National Institutes of Health (NIH)

**P50**

## Relationship between genome and transcriptome variation and the predictive capacity of the pan transcriptome to the pan genome

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Recent studies in plants and animals continue to advance our understanding of the relationship between genome content variation and phenotypic diversity. RNA sequencing has been used extensively to characterize genome diversity in a number of large plant genomes by using the transcriptome as a proxy for genic regions of the genome. This approach offers a number of advantages over whole genome resequencing, namely the ability to generate highly informative reads at a significant reduction in cost. Even so, it remains unclear how predictive the transcriptome is of genic content variation. To address this gap, we are using whole genome resequencing from 35 diverse inbred lines and RNA-sequencing-derived transcriptome data from these lines to evaluate the predictive capacity of the transcriptome in five diverse tissues (root, shoot, endosperm, internode, and leaf) for genic content variation. Beyond contributing to our knowledge of the relationship between the transcriptome and genome in maize, this research will also serve as a valuable resource in studies of non-model species for which few genomic resources exist.

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

**P51**

# Sequence, assembly and annotation of the maize W22 genome

(submitted by Thomas Brutnell <tbrutnell@danforthcenter.org>)

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Since it's adoption by Brink and colleagues in the 1950s and 60s, the maize W22 inbred has been utilized extensively to understand fundamental genetic and epigenetic processes such recombination, transposition and paramutation. To maximize the utility of W22 in gene discovery, we have Illumina sequencing technologies and de novo assembled a color-converted W22 reference genome. Significant structural heterogeneity exists in comparisons to the B73 reference genome at multiple scales, from transposon composition and copy number variation to single nucleotide polymorphisms. The generation of this high quality reference genome has enabled the accurate placement of several thousand Mutator and Dissociation transposable elements from community transposon mutagenesis projects that enable the use of both engineered and endogenous elements for reverse and forward genetics in maize. Annotation of the genome has been informed by RNAseq analysis, differential nuclease sensitivity profiling and bisulfite sequencing to fine map open reading frames, open chromatin sites, and DNA methylation profiles, respectively. Several independent assessments of the quality of the genome assembly including comparisons to high resolution genetic maps, Sanger-sequenced regions of high complexity and transposon site insertion mapping coupled with the integration of global epigenetic and gene expression annotation reveal that the W22 genome now represents the highest quality maize reference genome in the public domain.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Enterprise Rent-A-Car Institute for Renewable Fuels at the Danforth Plant Science Center

**P52**

## Sequencing, Assembly, and Annotation of Maize B104 : A Maize Transformation Resource

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Maize transformation is complicated. Most lines are not readily cultured and transformed, making the germplasm available for genome engineering extremely limited. Developing a better understanding of the genomic regions responsible for differences in culturability and transformability would be a good start toward enabling the development of new transformation strategies. B104 was derived from the same populations as the B73 reference. The genomes are very similar (93% similar as defined by Romay et al. Genome Biology 2013), but B104 is readily transformed whereas B73 is not. The availability of the B104 genome sequence provides an opportunity to create the genomics data infrastructure that would enable other researchers to work with their own transformants more easily. This poster outlines our progress towards these goals.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P53**

## The free and open-source AGB platform for managing genetics and breeding research programs

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We present Accrete Genetics & Breeding (AGB), a laboratory information management system to store and manage information on the plans, processes and data output of a genetics and breeding program in a systematic and easy to use way. AGB provides a complete workflow for managing breeding projects (from selecting stocks for planting to inventorying seed from the harvest). AGB also provides an active memory of the workflow for different projects and allows multiple collaborators to share/transfer responsibility in executing breeding and trialing activities across seasons and locations. This system is fully integrated in the Eclipse programming environment and is platform-independent. It consist of a user-friendly interface which is developed in Java Swing combined with Spring and Hibernate frameworks and a MySQL database plus R statistical computing back-end. The interface was designed based on modules. Without creating a project, users can manage information for the seeds that come from collaborators or collections, using an *Outside Seed* module; browse current and historic information via *Stocks Information*, *Preplanting Information*, and *Phenotype Information* modules; query, modify or create inventory information through an *Inventory* module. In addition, using the project manager and workflow, users will advance through modules to choose germplasm (*Stock Selection* module), create experimental designs with the use of R agricolae package (*Experimental Design* module), organize and plant genetic/breeding stocks and produce row or plant tags (*Planting* module), create data collection template files and upload collected phenotype or weather data (*Observation* module), and keep track of information like the types of mating and pedigrees, and create and catalogue harvested seed stocks (*Harvesting* module).

Funding acknowledgement: United States Department of Agriculture (USDA)

**P54**

## Tools for leveraging GWAS dat afor knowledge discovery

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We have developed two tools to help mine data from complex, multidimensional GWAS datasets: ZBrowse and the Comparative Genomics Association Study tool (CGAS).

ZBrowse is an interactive GWAS results viewer that is an extension of the classic GWAS Manhattan Plot. Zbrowse allows for the rapid graphical comparison of GWAS experiments performed on complex traits such as multiple phenotypes measured in multiple locations. The manhattan plots are fully interactive and results can be filtered to only display overlapping QTL between experiments. In addition, results with base pair ranges, such as joint linkage support intervals, can be viewed on the same plot as the GWAS results to quickly visualize overlaps. The browser allows for easy and interactive navigation between plots displaying the entire genome, down to a plot less than a mega base wide displaying gene tracks.

The focus of CGAS is to leverage the power of GWAS with comparative genomics approaches. CGAS allows users to compare GWAS results between organisms to find homologous genes under loci associated with a phenotype of interest. Using a set of SNPs or genetic intervals from two organisms as input, CGAS find genes within the intervals and uses phytozome homolog tables to find homologous genes. To assess the statistical significance of CGAS results, we have developed a permutation algorithm to calculate the background probability of finding overlapping genes in similar, randomly generated datasets.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P55**

## Transcriptome profiling and regulatory network analysis of maize aleurone and starchy endosperm cells at different developmental stages

(submitted by Xinxin Ding <xding4@wisc.edu>)
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The cereal endosperm consists of starchy endosperm (ST) cells, which accumulate storage proteins and starch, the peripheral aleurone (AL) cells, which mobilize these storage compounds during germination, and transfer cells (BETL), which are in contact with the embryo. By analyzing the genes expressed in maize ST and AL as well as BETL, embryo (EMB) and embryo surrounding region (ESR) at different developmental stages, we want to identify main metabolic pathways and regulatory networks related to both development and nutritional properties of the maize endosperm. Here we present a systematic comparison and analysis of the transcriptomes of AL, ST, EMB, BETL, and ESR at 8, 15, 18 and 22 days after pollination (DAP). A total of 2,945 differentially expressed genes were identified by comparing AL and ST at 18 and 22 DAP. Twenty one coexpression modules were identified for 10,238 genes in the 14 different kernel tissue samples. The modules contain genes with distinct expression patterns related to both developmental stages and tissue types. We performed gene ontology enrichment tests and found that the modules contain genes involved in different biological processes, most importantly on biosynthesis of pigments and flavonoid, amino acid metabolism, Golgi vesicle and vacuole related transport, brassinoisteroid signaling, and sugar-mediated signaling. We also applied modular regulatory network learning with per gene information (MERLIN) to the 10,238 genes and predicted regulatory networks involving transcription factors and co-regulators for some of the identified modules.

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**P56**

## Transcriptomic Analyses of Maize-Southern Corn Leaf Blight Interaction

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Southern Corn Leaf Blight (SLB) is a foliar disease caused by the fungus, *Cochliobolus heterostrophus* (aka, *Bipolaris maydis*). Under warm and wet conditions in temperate production areas, crop losses of up to 70% have been blamed on this disease. Breeding has produced disease resistant cultivars that have been effective in limiting losses in recent years, but additional research is needed to ensure vigilant crop protection over the longer term. One of our primary objectives is to understand the molecular basis of quantitative disease resistance in maize. To this end, we performed transcriptomic analyses across histologically defined stages of SLB pathogenesis in B73 and Mo17, which contrast moderately resistant versus moderately susceptible disease progressions that are not masked by qualitative resistance factors. Using a split-split plot design with 4 complete replications, pooled samples of inoculated or mock-inoculated 5th leaf tissue were collected at 12, 24 and 48 hours after treatment from both genotypes. RNAseq (100 bp PE) reads were aligned to the B73v3 genome and counted for differential gene expression analyses using ensembl release No. 28 GFF3 coordinates. Unmapped reads were *de novo* assembled using Trinity. Resultant contigs were mapped to the genome of SLB_isolate C5, as well as to known Mo17 and B73 transcripts. DE analyses for both the host and pathogen transcriptomes have been conducted. For the maize transcriptomes, we enumerate DEGs according to treatment, host genotype, and timepoint, and can distinguish circadian versus disease responses within timepoint DEGs by comparing SLB- versus mock-inoculated samples. For the SLB transcriptomes, we are able to examine how the host genotype strongly impacts gene expression within individual time points. In both studies, we are pursuing pattern analyses methods to infer trends with biological meaning across the time course.

Funding acknowledgement: National Science Foundation (NSF)

**P57**

## Translational dynamics of nuclear genes during leaf development in maize

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The strap-like maize leaf develops from the tip to the base, providing an ideal opportunity to monitor the process of photosynthetic differentiation. In recent years, both RNAseq and proteomics studies have been utilized to survey this dynamic process but both methods suffer limitations. In RNAseq analysis, non-translated transcripts have the potential to bias estimates of gene expression, whereas in proteomic surveys, reduced sensitivity of this technique relative to RNAseq prevents direct correlations of transcript to protein profiles. Ribosome profiling (Ribo-seq), a relatively new technique, is a deep-sequencing based method that provides a global view of translation at any given point of time. This technique is based on sequencing ribosome protected mRNA fragments that are used as a proxy for translation. Thus, the technique provides an opportunity to exploit high throughput sequencing technologies to simultaneously estimate both transcript and protein levels. Here, we present our results of Ribo-seq along a developing leaf gradient of maize. We have optimized a computational pipeline to analyze Ribo-seq data from maize and present our preliminary data that suggests that the translational efficiency of several thousand genes changes 4 fold or more during the course of photosynthetic differentiation.

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**P58**

## Updated grass syntenic gene sets

(submitted by James Schnable <schnable@unl.edu>)
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Syntenic orthologs are known to be more likely to play conserved functional roles across species. However, becoming apparent that maize genes with syntenic orthologs in one or more related species are significantly more likely to show evidence of playing any functional role than non-syntenic genes which duplicated or transposed into their present locations in the maize genome more recently. Previous work demonstrated that genes responsible for qualitative (ie mendelian mutant) phenotypic variation in maize are more likely to be conserved at syntenic locations in the genomes of other grass species. New data shows that the same trend of genes conserved at syntenic locations in multiple species being more likely to identified as the underlying cause of phenotypic variation also extends to quantitative phenotypic variation. We also find the increase in gene density on euchromatic arms is predominantly driven by syntenically conserved ancient genes, while non-syntenic genes show a largely even density in terms of genes/MB across the maize genome. Finally we demonstrate how comparisons across multiple species can be used to identify gene models likely to have been incorrectly split or merged during the annotation process.

We have updated and extended syntenic orthologous gene lists originally published in 2012 to use the most recent releases of the maize and sorghum reference genomes (B73 RefGen V3 and v3.1 respectively) as well as extending our dataset to include data on syntenic orthologs of maize genes in the recently published reference genomes for *Setaria italica*, *Oropetium thomaeum*, and *Dichanthelium oligosanthes*. These updated syntenic gene sets are being made available to the community with documented methodology and a citable DOI on FigShare prior to publication.

**P59**

## Updates to qTeller: A tool for visualizing published gene expression data.

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Over the last five years, RNA-seq data has become cheaper and more affordable than ever. However, for the vast majority of researchers engaged in genetic, as opposed to genomic, research it is still more useful to be able to track the expression of individual genes, or a set of candidate genes in a mapping interval, across many different tissues and conditions than to study the expression of every gene in the genome across a small number of tissues or conditions. So, qTeller was developed to provide researchers with a zero-cost and easy-access tool to use published RNA-seq datasets to provide exactly that type of data. The qTeller website uses published RNA-seq datasets from different studies by research groups around the world to provide information on the expression pattern of specific genes in a wide range of growing conditions and in different tissues and cell types. Based on the expression values of individual genes, researchers are often able to narrow down their list of candidate genes within a mapping interval.

qTeller originally came online in 2012. However, since that time additional datasets have been published, and more critically, new versions of both the maize and sorghum genomes have been released, substantially reducing the utility of this tool for researchers. By going back to the original raw sequence data and reprocessing it I have developed new databases for both the maize and sorghum qTeller instances using the latest genome release versions (B73_RefGen_V3 and v3.1 respectively). These new qTeller releases also include more paired datasets generated from sorghum and maize plants grown under identical conditions with the hope of increasing the comparability between the maize and sorghum qTeller instances.

Funding acknowledgement: UNL Start-up Funding

**P60**

## Why Principal Components can Separate Teosinte, Landraces, and Improved Varieties?

(submitted by Xianran Li <lixr@iastate.edu>)
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Maize genomes harbor over 9 million common SNPs. The genome divergence among teosinte, landraces, and improved varieties can be captured by a few of principal components derived from genome-wide SNPs, which is another demonstration for applying principal component analysis in population genomics. However, the underlying reason for principal components (PCs) have this ability remains unknown. One impediment is that the mathematic operation ascribes no biological bearings to PCs. A biological meaningful measurement, Base Composition across SNPs (BCS), can be derived from genome-wide SNPs, as teosinte has lower AT content than maize across all SNPs. Our empirical observation and theoretical derivation indicated that the first PC (PC1) is correlated with this measurement. Therefore, we hypothesized that leveraging BCS can address this question.
Among 9 million SNPs, 2 million are nearly fixed in teosinte, which implies that the new allele was introduced in maize after the domestication process. PC1 is highly correlated with BCS from these 2 million SNPs, indicating that PC1 aggregates information from this set of SNPs to separate teosinte from maize. On the other hand, standing alleles from wild ancestors might pass through the domestication bottleneck and enriched their frequencies at different levels in maize. We found PC2 extracts information from this type of SNPs (3 million) to make the separation among maize, as indicated by the correlation with BCS. Lastly, modern breeding programs further enriched some alleles in improved varieties, and PC3 combines information of 0.6 million SNPs selected by breeding process and separates landraces from improved varieties. Similar biological interpretation of PCs was obtained with Human 1000 Genomes data. Forces of evolution including mutation, drift, gene flow, and selection have different impacts on genome divergence. Our analysis suggested that individual PCs captures one unique genome divergence footprint, and collectively, reveal the overall evolution pattern.

Funding acknowledgement: National Science Foundation (NSF)

**P61**

## A forward genetics approach to explore natural variation for enhancer/suppressors identifies components of the guard strategy of plant immunity

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The focus of this poster is Slm1 (suppressor of lesion mimics-1) and its target mutation, les23. Slm1 was discovered as a QTL in a natural enhancer/suppressor screen named MAGIC, for Mutant-Assisted Gene Identification and Characterization. The reporter mutant phenotype used in this study was provided by les23, a recessive lesion mimic mutation that results in symptoms resembling hypersensitive cell death. To understand how Slm1 may suppress les23, we cloned and confirmed the gene responsible for Slm1 by a combination of approaches involving positional cloning, transposon tagging with Mutator (Mu) and directed mutagenesis with EMS. The results revealed Slm1 to be a defective R gene. To determine how an R gene encoding a truncated protein could act as a suppressor of les23, we cloned the gene underlying this mutant. A missense mutation leading to single amino acid substitution in a homolog of the Arabidopsis RIN4 gene was found to cause the les23 mutation. Originally identified as an interactor of the R protein RPM1, RIN4 has emerged as a key component of the guard mechanism of plant innate immune responses. Degradation of RIN4 or a loss of physical association with the R proteins RPM1 or RPS2 leads to a robust hypersensitive cell death response. In this regard, it is possible that the mutation in les23 causes a conformation change that results in loss of interaction with Slm1 and induces HR. However, if Slm1 is non-functional, as it is in the les23-suppressing QTL Slm1, no cell death is initiated whether the maize ZmRin4 is defective or not. Surprisingly, we found that Slm1 does not appear to be highly conserved among the NAM founders as only 5 lines are completely identical to the B73 sequence while 7 lines contain mutations that result in a truncated protein.

Funding acknowledgement: National Science Foundation (NSF)

**P62**

## A Genome Engineering System in Plants via Intra Genomic Homologous Recombination and Nuclease-Mediated Cassette Exchange

(submitted by Stephen Novak <snnovak@dow.com>)
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To meet the need for durable, broad-spectrum control of weed and insect pests in different geographies, modern agriculture requires the use of multiple transgenes and the flexibility of combining them with new traits as they are developed. We recently developed a gene targeting platform for creating linked, multi-gene stacks. The method utilized a unique intron sequence inserted directly downstream of a promoter controlling the expression of a selectable marker in a donor sequence and its use in homology-directed repair for Nuclease-Mediated Cassette Exchange (NMCE) between target and donor. We now further extend this gene targeting method to convert a transgene stack containing two unlinked trait loci into a single locus stack. The method utilizes intra-genomic homologous recombination (IGHR) between stably integrated target and donor loci, which share sequence homology and nuclease cleavage sites whereby the donor contains a promoterless herbicide resistance transgene. Upon crossing with a zinc finger nuclease (ZFN)-expressing plant, double strand breaks (DSB) are created in both the stably integrated target and donor loci. DSBs flanking the donor locus result in intra-genomic mobilization of an excised, selectable marker-containing donor sequence, which can be utilized as a template for homology-directed repair of a concomitant DSB at the target locus. The method was successfully demonstrated in maize and up to 3.3% of the resulting progeny embryos cultured on selection medium regenerated plants with the donor sequence integrated into the target locus. The method could be extended to multiple cycles of trait stacking by virtue of a unique intron sequence homology for NMCE between the target and donor loci. This is the first report that describes NMCE via IGHR thereby enabling trait stacking using conventional crossing.

Funding acknowledgement: Dow AgroSciences LLC

**P63**

## A phylogenetic framework for characterizing CYP72A enzyme function in secondary metabolism

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The genetic resources for maize make it an excellent system for studying metabolic responses to insect damage. Cytochrome P450 monooxygenases (CYPs) are enzymes that have been implicated in many aspects of secondary metabolism, but much is still unknown about the biochemical capabilities of this large class of enzymes, particularly in the grasses. Plant genome sequencing has revealed the presence of thousands of CYP genes with an average of about 300 genes per plant. The CYP72A subfamily appears to have members in all angiosperms and provides the potential for a variety of biochemical functions in each plant species. There are eleven genes for CYP72A enzymes in maize in clusters on chromosome 3 and chromosome 8. Aphid and/or caterpillar feeding induces gene expression for some of the CYP72As. Most of the maize CYP72A sequences have orthologous partners in sorghum and several have orthologous relationships with sequences from rice and *Brachypodium*. Phylogenetic relationships and gene expression data provide a hierarchy of relative contributions of each of the CYP72A enzymes in maize. Using this hierarchy, we have begun isolating Mu and Ds insertion mutants in CYP72A genes. Metabolic profiling suggests that CYP72A349 (GRMZM2G123309) plays a role in constitutive metabolism. We are further examining the contributions of CYP72A enzymes in induced defenses. These data contribute to a better understanding of the metabolic potential of the CYP72A subfamily in maize and related grasses.

Funding acknowledgement: National Science Foundation (NSF)

**P64**

## A preliminary study of the function of Zma-miR159c in grain filling

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Zma-miR159c was repressed in the endosperm during maize grain filling, which hinted that it might be one of the functional miRNAs regulating maize grain filling. To investigate the function of zma-miR159c, the Arabidopsis and rice transgenic systems were used. The function of zma-miR159c and its target gene in Arabidopsis, At-MYB33 was verified by suppressing zma-miR159c with the short tandem target mimic (STTM) method, overexpressing Pre-miR159c, and mutating At-MYB33 with clustered regularly interspaced shot palindromic repeats (CRISPR). The results showed in STTM159c transgenic plants, seed length and seed weight were decreased compared to wide type, and in Pre159c and CRISPR-MYB33 transgenic plants, seed length and seed weight were increased. In rice system, Over-expression of zma-miR159c also showed an increasing seed length and seed weight phenotype. Degradome data shows that the target genes of zma-miR159c in rice encoded a MYB transcriptional factor and a large subunit of 60S ribosomal protein. RNA-seq of transgenic and wide type rice endosperm showed that zma-miR159c may influence rice grain filling directly by enhancing the second cell wall synthesis pathway and decreasing the starch catabolic pathway; and via indirectly influencing mRNA transcription and processing.

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**P65**

## Accurate Calibration Transfer between NIR Spectrometers for Prediction of Single Seed Composition and Shape Traits

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Single seed near infrared reflectance (NIR) spectroscopy predicts maize and soybean seed quality traits such as oil or protein. We tested the accuracy of transferring calibrations between different single seed NIR analyzers of the same design by collecting NIR spectra and analytical trait data for globally diverse germplasm. X-ray micro-computed tomography (μCT) was used to collect seed density and shape traits to enhance the number of soybean traits that can be predicted from single seed NIR. Partial least squares (PLS) regression gave accurate predictive models for oil, weight, volume, protein, and maximal cross-sectional area of the seed. PLS models for width, length, and density were not predictive. Although principal component analysis (PCA) of the NIR spectra showed that black seed coat color had significant signal, excluding black seeds from the calibrations did not impact model accuracies. Calibrations for oil and protein developed in this study as well as earlier calibrations for a separate NIR analyzer of the same design were used to test the ability to transfer PLS regressions between platforms. PLS models built from data collected on one NIR analyzer had minimal differences in accuracy when applied to spectra collected from a sister NIR analyzer device. Furthermore, model transfer was more robust when spectra were trimmed from [910-1679 nm] to [955-1635 nm] due to divergence of edge wavelengths between the two NIR spectrometer devices. The ability to transfer calibrations between similar single seed NIR spectrometers facilitates broader adoption of this high-throughput, non-destructive, high accuracy seed phenotyping technology.

Funding acknowledgement: National Science Foundation (NSF)

**P66**

## Allelic variations for key provitamine A genes in maize

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Carotenoid intake plays an important role in human nutrition and health owing to the association of their consumption levels with reduced risk of diseases such as cardiovascular disease, cancer, and age-related sing problems arising from deficiencies of lutein and zeaxantine. Maize can naturally accumulate both provitamin A and non-provitamin A carotenoids in its kernel. Provitamin A constitutes only 10 to 20 % of the total carotenoids in maize kernel. The predominant carotenoid in maize kernels, in decreasing order of concentration, are lutein, zeaxanthin, β-carotene, β-cryptoxanthin and α-carotene. β-carotene contains two provitamine A structures (two non-hydroxylated β-ionone rings) and β-cryptoxanthin and α-carotene one each (single non-hydroxylated β-ionone rings). Specific nucleotide sequence variants within the key carotenogenic genes have also been characterized, and shown to contribute significantly to accumulation of provitamin A and total carotenoids in maize endosperm. Favorable alleles possessing rare genetic variation in lycopene-ε-cyclase (lcyE) and β-carotene hydroxylase (crtRB1) gene are associated with higher accumulation of provitamin A.
We tested two of the three significant polymorphisms lcyE (SNP216 and 3' indel) and 3' TE region of crtRB1 genes. Screening of a diverse set yellow maize inbred lines detected the presence of one alleles of lcyE (amplicon size 144 + 502 bp) and three alleles of crtRB1 (amplicon size: 296, 543 and 296 + 875 bp). The high provitamin A inbred lines harboring combinations of the favourable alleles of the crtRB1 and lcyE markers can be used to speed up the development of next generation of high provitamin A maize hybrids.

Funding acknowledgement: National academy of agrarian sciences of Ukraine

**P67**

## An *empty pericarp* phenotype results from mutation of a nuclear-encoded group II intron maturase implicated in splicing of mitochondrial transcripts in maize

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Mitochondria have their own genome, which contains numerous group II cis/trans introns. The mechanism of processing pre-mRNA in mitochondria differs from that of most nuclear introns. The removal of group II introns does not require participation of the large complex spliceosome. Instead, a maturase protein directly binds the group II intron of pre-mRNA to facilitate its folding and splicing. In bacteria and yeast, the splicing of group II introns is facilitated by maturase proteins encoded within the introns themselves. However, only a single maturase gene (*MatR*) remains in the plant mitochondrial genome, the other maturase genes having moved to the nuclear genome during evolutionary history. In maize, we have identified five members of the type II intron maturase family in the nuclear genome (*Mat1, Mat2a, Mat2b, Mat3 and Mat4*). They are predicated to target to the mitochondria, where we hypothesize that they play roles in splicing the group II introns. The protein sequences of Mat2a and Mat2b are highly similar, differing only in nine amino acids. By screening the UniformMu population, we obtained transposon-insertion mutants for these genes. Among them, the *mat1* mutant has an *empty pericarp* (*ep*) phenotype. Genetic allelism tests between multiple mat1 alleles indicated that loss of *Mat1* function was responsible for the *ep* phenotype. Although kernels are infertile and the phenotype is dramatic, both endosperm and embryo progress through initial stages of development without critical aspects of mitochondrial function.

Funding acknowledgement: National Science Foundation (NSF)

**P68**

## Analysis of drought stress response mechanisms of two elite maize lines using RNA-Seq

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Drought is one of the major constraints of plant productivity worldwide. Understanding genetic and molecular mechanisms underlying the diverse strategies of drought tolerance is required in breeding and genetic engineering of drought tolerant plants. We phenotypically characterized two elite maize lines, which are the parents of a comprehensive introgression library, under well watered and drought conditions in the greenhouse. The recurrent parent (RP) is highly adapted to drought prone areas in Southeastern Europe while the donor parent (DP) is not drought tolerant. For RP a decrease in stomatal conductance and consequently also in the photosynthetic rate was observed earlier than for DP. Our objective is to compare the drought stress response between RP and DP on the molecular level. To this end we performed time-series RNA-Seq analyses of leaf tissue under mild, medium and severe drought stress and after stress recovery for both genotypes. We identified differentially regulated genes (DEGs) under stress conditions for both lines and compared the DEGs between lines and sampling times. We found that genes associated with photosynthesis were very early downregulated in RP compared with DP, which is in line with RP's early response of stomata closure. Conversely, genes encoding heat shock proteins were much stronger regulated in DP compared with RP, indicating that the two maize lines have evolved different drought adaptation mechanisms. In addition to protein coding genes we also deep-sequenced small RNAs and identified microRNAs for both genotypes that were differentially regulated under drought stress. Latest results on expression analyses of candidate genes for drought tolerance will be presented.

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**P69**

## Application of a Functional Gene Discovery Platform to Identify Novel Acyanogenic Sorghum Lines

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Sorghum is one of the major cereal crops in the world, ranking fifth in global cereal production with a most recently recorded total of 67,870,661 t in 2014 (FAOSTAT 2014). Besides its use for human consumption, sorghum is an important source of forage biomass and is used as silage for livestock. A major obstacle to the use of sorghum for animal feed is the presence of the cyanogenic glucoside dhurrin in the shoots of plants. Dhurrin is present at high concentrations in shoot tips of sorghum seedlings, but decreases when plants become older. A concentration greater than 0.1 percent of dry tissue is considered highly dangerous and seedlings can accumulate dhurrin of up to 6% of their dry weight. When tissues containing dhurrin are consumed, hydrogen cyanide (HCN), also known as prussic acid, is released during the process of dhurrin decomposition. Here, we demonstrate the application of a functional genomics platform, created with the support of the Bill and Melinda Gates Foundation (BMGF), to identify point mutations in genes coding for the enzymes CYP79A1 (biosynthesis) and dhurrinase 2 (catabolism). Two knockout mutations for each gene were assessed for dhurrin production and correlations with agronomic performance, biomass accumulation and grain quality. Preliminary results suggest the mutants genotypes do not differ significantly in biomass accumulation traits.

Funding acknowledgement: Bill & Melinda Gates Foundation

**P70**

# Application of fluorinated sucrose derivatives to study phloem transport in maize

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Phloem tissues play a central role in the transport of photoassimilates from source tissues to sink tissues. However, there are many challenges in the study of phloem transport (for example, phloem transport is very sensitive to physical damage). What is needed is a way to overcome these challenges, and one possibility is the use of radiotracer imaging. Here we demonstrate the novel synthesis and use of fluorinated sucrose derivatives as new radioactive tracers to study phloem transport in maize leaves.
Sucrose derivatives were individually fluorinated with the positron emitting radioactive isotope fluorine-18 at the 1' or 6'carbon positions within fructose, or the 6 carbon position within glucose. To test whether these derivatives can be transported through the phloem, each derivative was independently introduced into the leaf tips of wild-type plants and plants that lacked *Sucrose transporter1 (Sut1)*, which was previously shown to function in sucrose phloem loading. The plants were incubated for an hour, then dissected leaves were imaged using autoradiography.
All three fluorinated sucrose derivatives were able to be translocated down the leaf blades, indicating that they are substrates for phloem loading. The mutants exhibited significantly less transport of the derivatives than the wild-type plants. In addition, the three derivatives were similarly transported, suggesting that these specific fluorinated positions do not interfere with sucrose binding by the SUT1 protein or affect sucrose transport across the phloem cells plasma membrane. Our study indicates that these fluorinated sucrose derivatives can be successfully used for the in vivo study of sucrose transport in maize.
In future research, we will apply the fluorinated sucrose derivatives combined with dynamic radiotracer imaging techniques, especially positron emission tomography (PET). This will enable the investigation of photoassimilate transport in living plants in real time as well as under different abiotic stress conditions.

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**P71**

## Balancing the source and sink tissues for increased grain methionine content in maize

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Zeins, the predominant seed storage proteins in maize, account for more than 50% of the total seed protein and are deficient in the essential amino acid methionine. The majority of maize production goes to animal feeds and as a result, maize is supplemented with synthetic methionine to overcome its nutritionally poor status. Here we present two approaches that we have used to increase grain methionine content in maize. Both use alterations in either the source (e.g., leaf) or sink (e.g., seed) tissues, thereby affecting accumulation of the 10-kDa δ-zein, which is the main reservoir of methionine in the grain. Repression of 10-kDa δ-zein levels has been overcome by transgenic means. However, the increase in the 10-kDa δ-zein occurred at the expense of the sulfur-rich 15-kDa β- and 16-kDa γ-zeins due to limitations in sulfur availability. To address the limitations in sulfur availability, we applied the leaf-specific (through *PepC* and *RbcS* promoters) expression of the *Escherichia coli* 3'-phosphoadenosine-5'-phosphosulfate reductase (*Ec*PAPR), a regulatory enzyme of the sulfate assimilation pathway. Interestingly, now the increase in the 10-kDa δ-zein levels does not lead to decreased accumulation of the 15- and 16-kDa zeins, illustrating that sulfur availability represents the bottleneck for grain methionine accumulation. Transgenic lines with both transgenes have patterns of zein accumulation similar to that of the 10-kDa δ-zein overexpressor, suggesting a threshold to methionine accumulation in the maize grain. The level of methionine in the 10-kDa δ-zein overexpressor approaches that of B101, a high-methionine maize inbred, whereas *Ec*PAPR-expressing plants have levels exceeding that of B101. Currently, the nutritional values of the *Ec*PAPR-expressing plants are being evaluated.

**P72**

## Carbohydrate Analysis of Five Independent Mutations at the *su1* locus

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*Sugary1 (Su1)* codes for isoamylase1, a debranching enzyme required for the production of amylopectin in the endosperm. Mutants at *Su1* result in either no protein or inactive proteins. As a result, amylopectin production is reduced and water-soluble polysaccharides (WSP) are produced. We backcrossed five naturally occurring *su1* alleles derived from five independent origins: northeastern, north central, southwestern, Central Mexico, and Peru, into two field corn inbreds A619 and A632 (7 backcrosses). We evaluated the carbohydrate composition of mature kernels in, F1 and F1 reciprocal crosses. The F1 and reciprocal seed was planted in 2015 in a randomized complete block design with two replications in two environments. At maturity, starch and WSP concentrations were measured. The northeastern by northeastern hybrid had significantly greater WSP and lower starch concentrations than all other hybrids, and most closely resembled concentrations found in commercial sweet maize. The Peruvian by Peruvian hybrid had significantly lower WSP and greater starch concentrations than all other hybrids, and resembled concentrations found in wild type maize. The remaining hybrid combinations suggest that a dosage effect is associated with the northeastern and Peruvian alleles. All of these alleles with the exception of the Central Mexican produce a nonfunctional isa1 protein. Two possible explanations for the observed differences include unknown protein-protein interactions or the presence of other loci affecting starch synthesis linked to the backcrossed alleles.

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**P73**

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

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Mutant, an important material for both classical genetics and functional genomics research, plays a significant role in the functional annotation of genes and the interpretation of biochemical and metabolic pathways in plants. In this study, a spontaneous seed color mutant of maize, *slcd* (seed and leaf color defective), was identified and characterized by the light yellow grain and the albino plant, which results to the death of the whole plant. Cloned by map-based cloning and confirmed by allelism test, transgenic functional complementation and RNA-knock out, the *slcd* was detected to have a 7608-bp insertion of a gypsy-like LTR retrotransposon in intron 8, which results in the loss of function of this gene by producing alternative transcript splices. The *SLCD* gene encodes IspG, an HMBPP Synthase catalyzing the conversion of methylerythritol 2,4- cyclodiphosphate to hydroxymethylbutenyl diphosphate (HMBPP), which is the penultimate step of the MEP pathway. The *slcd* mutant exhibits impaired chloroplast in leaf and affects the endomembrane system in the developing endosperm. RNA-seq analysis of 14DAG seedlings and developing endosperm (15DAP) provides molecular insights into the regulatory mechanisms of *SLCD* in both leaf and endosperm. These results expand the current understanding of the MEP pathway regulatory mechanisms in plant development.

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**P74**

## Characterization of *Carbohydrate Partitioning Defective2*

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Carbon fixation assimilates inorganic carbon into carbohydrates, which provide energy for plant growth and development. Consequently, the plant needs to transport soluble sugars, largely in the form of sucrose, from photosynthetic source tissues (leaves), to non-photosynthetic sink tissues (roots, seeds, fruits, etc.). When carbohydrate partitioning, the distribution of fixed carbon throughout the plant, is disrupted it results in an increase of sugars and starch within leaves. We performed genetic screens for plants displaying leaf chlorosis, anthocyanin and starch accumulation in leaves, and overall decreased plant growth to identify *carbohydrate partitioning defective (cpd)* mutants. One such mutant, *Cpd2*, is a semi-dominant mutant that exhibits these phenotypes. The mutant was initially mapped to the short arm of chromosome four. Fine-mapping is currently being used to narrow the position of the mutation responsible for these phenotypes. Identification and characterization of the causative mutation will give insight into the gene's role in carbohydrate partitioning. Understanding this crucial transport pathway will help guide genetic improvements for C4 grasses, and enhance our knowledge of metabolic pathways.

Funding acknowledgement: National Science Foundation (NSF)

**P75**

## Characterization of cross-incompatibility

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Pollen-mediated gene flow from genetically engineered (GE) corn varieties into non-GE varieties causes adventitious presence, thus affecting co-existence and organic grain production. Differences in market value between GE and non-GE grain requires each market class to remain distinct with several agricultural practices currently being utilized to achieve purity standards. Presently, spatial separation of GE and non-GE maize is the most reliable practice for reducing cross-contamination but a biological barrier, like *Gametophyte factor1-strong (Ga1-s)*, may be a more effective means to reduce adventitious presence with its ability to exclude GE pollen from non-GE varieties if deployed correctly. Although several mapping studies for *Ga1-s* have been completed, a causative gene has yet to be identified. Our studies seek to understand the biochemical nature of *Ga1-s* by characterizing pollen tube growth under *in vitro* and *'in vivo'* conditions. A significant difference in growth rates was found between pollen tubes carrying the *Ga1-s* allele (compatible) and those without the allele (incompatible) when *Ga1-s* was present in the female parent. Incompatible tubes not only grew at 30% of the normal rate but also experienced aberrant growth with tubes exiting and re-entering the transmitting tract. A proteomics analysis of silk tissue revealed four proteins of interest. One protein, a cytochrome P450 enzyme, has a gene model close to the region of interest identified by previous mapping studies. In conclusion, differences in pollen tube growth caused by the *Ga1-s* allele may be due to a protein factor expressed in maize silks. Future work is needed to determine the presence of this protein in other genetic backgrounds introgressed with the *Ga1-s* allele.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P76**

## Characterization of targeted missense Zea mays Histidine Kinase1 mutations in *Saccharomyces cerevisiae* reveals residues important for signaling activity

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Cytokinins (CKs) regulate a diverse assortment of processes in plants, including cellular division, vasculature differentiation, and meristem maintenance. CK perception and response is regulated through a two-component signal transduction system consisting of histidine-kinase receptors, histidine phosphotransferase proteins and response regulators. Two-component signaling systems are highly conserved in bacteria, fungi and plants and allow organisms to sense and respond to diverse stimuli. *Zea mays Histidine Kinase1 (ZmHK1)*, a CK receptor, was identified as the gene underlying the semi-dominant mutant *Hairy Sheath Frayed1 (Hsf1)*. *Hsf1* plants are marked by outgrowths of proximal leaf tissue (sheath, auricle, and ligule) in the distal leaf blade, reduced leaf size, and increased leaf pubescence. Three specific missense mutations in the CK binding domain of ZmHK1 define all the *Hsf1* alleles identified to date. Each mutation causes increased ligand binding affinity and CK hypersignaling, giving rise to the *Hsf1* phenotype. Using a *Saccharomyces cerevisiae* reporter strain where the endogenous his-kinase receptor gene has been functionally replaced by plasmid derived copies of the heterologous *ZmHK1* gene, we tested the ability of a series of 18 targeted missense ZmHK1 mutations generated specifically in the ligand binding domain to promote *Hsf1*-like CK hypersignaling. Yeast harboring these different mutant *ZmHK1* genes were grown with and without different CKs to quantitatively analyze their ability to bind and signal. Some mutations led to Hsf1-like hypersignaling, while others showed no significant changes in activity or completely disrupted signaling. These targeted amino acid changes are providing insight as to which residues are critical for ligand recognition, binding, and signaling. Our current characterization and analysis will be presented.

Funding acknowledgement: National Science Foundation (NSF)

**P77**

## Cloning and characterization of *roothairless 6* in maize

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Root hairs are important for water and nutrient acquisition by increasing the root surface. In maize (*Zea mays* L.), six mutants (*rth1 - rth6*) with impaired root hair elongation have been identified. Here we describe the mapping and characterization of the novel gene *roothairless 6* (*rth6*), which is involved in cell wall development.

*rth6* mutants display significantly shorter root hairs than their wild-type siblings. While root hair bulges are formed in these mutants, root hairs fail to elongate due to immediate ruptures. The *rth6* gene was mapped to the short arm of chromosome 1. The candidate gene was subsequently identified to be involved in cell wall development. In a phylogenetic analysis close homologs were identified throughout the plant kingdom and revealed conserved functions in cell wall formation. Many of these genes play a crucial role during polarized tip growth. The *rth6* gene is the only member of its gene family showing preferential expression in root hairs.

The *rth6* gene plays a crucial role in cell wall development during root hair growth. Modulation of genes related to root hair formation may enhance root hair growth for improved nutrient uptake.

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**P78**

## Comparative expression analysis of domesticated grasses and their wild relatives when exposed to saline conditions

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In 2011, the world's population was estimated at 7 billion, and by mid-century this figure is expected to reach 10 billion. In order to provide food, fuel, and fiber for this growing population, crop production is forced to migrate to lands with marginal soils where plants are less productive. One characteristic of marginal soils is increased salinity. Salinity increases as a result of irrigation, drought, ingression of seawater, and weathering of the Earth's crust. The exposure of the Earth's crust increases the solubility and abundance of ions, which yields higher salt content in the surrounding soil and water. With increased salinity, plants are less productive due to decreases in tillering, decreases in grain yield, and decreases in biomass. Therefore, salt tolerant genotypes are critical for managing the increased demand of crop production on marginal soils. In the work described here, we aim to delineate the gene expression responses of two domesticated and two wild grass species (Sorghum bicolor, Sorghum verticilliflorum, Setaria italica, and Setaria viridis) to salt exposure. Beginning at the five-leaf stage of development, plants were watered with increasing levels of sodium chloride solution. At nine weeks post germination, the fifth leaf was collected for RNA extraction and Illumina library construction. All libraries were sequenced on the HiSeq 1500 and differential gene expression was compared for salt and non-salt treatments. Genes that were significantly differentially expressed were further characterized using the Plant Metabolic Network, which reveals the role that genes play in different metabolic pathways. Our results provide insight into plant genomic responses to a common abiotic stress found not just on marginal lands, but also lands associated with global agricultural practices. In addition, these gene expression comparisons provide insight into the evolution of parallel domestication and lineage-specific pathways for salinity tolerance.

**P79**

## Comparing transcriptome responses to N in hydroponic and field experiments

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The positive growth response of maize ears to nitrogen is important to achieving high yields, but the underlying genetic and physiological mechanisms for this phenomenon are currently unknown. Many previous studies have investigated transcriptome responses to nitrogen in maize seedlings or young vegetative plants grown in liquid media, but the relevance of these regulatory programs to leaves and ears from field-grown plants has not been directly assessed. In cooperation with DuPont Pioneer, we grew the fast maturing maize line Gaspé Flint in a hydroponics system that permits addition of nitrogen to plants that have already initiated ears. This system was used to perform a nitrogen-induction experiment followed by RNA-Seq profiling just prior to induction, and either four or eight hours after nitrogen treatment. RNA-Seq profiles of leaves and developing ears from the hydroponics experiment will be compared to RNA-Seq profiles from similar tissues for B73 plants grown in the field with a continuously low or high soil nitrogen supply. Analysis of these profiles will reveal those aspects of the nitrogen regulatory network that are robust to, or variable, among the growth stages and conditions tested.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Pioneer Hi-Bred International

**P80**

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

(submitted by Robert Augustine <raugustine@wustl.edu>)
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Plants rapidly initiate a variety of cellular responses to cope with environmental challenges. Among the fastest is the stress-induced conjugation of small ubiquitin-related modifier (SUMO) to large collections of nuclear proteins involved in a diverse array of chromatin and RNA processing events. Despite the importance of SUMOylation to stress tolerance, little is known about the function(s) of this modification in crops. Here, *in silico* approaches were used to identify major SUMO pathway components in maize (*Zea mays*). This list includes three SUMOs, E1, E2 and E3 enzymes involved in the conjugation cascade, and an array of deSUMOylating proteases that reverse the modification. Phylogenetic analysis reveals that most plants express a divergent 'non-canonical' SUMO, which has likely evolved through multiple independent duplications, and a more ancient, highly-conserved 'canonical' SUMO with essential functions. Additional SUMOs include a conserved SUMO variant with an elongated, charged N-terminal extension followed by the signature *beta*-grasp fold, and a monocot-specific DiSUMO-like protein bearing two tandem SUMO-type, *beta*-grasp folds. The seven-member E2 gene family subdivides into a conserved group with constitutive expression, and a cereal-specific group whose expression is more restricted and has likely subfunctionalized. Assays using recombinant enzymes demonstrated the functionality of the maize SUMO machinery, and, like Arabidopsis, maize rapidly SUMOylates an array of proteins *in planta* upon heat stress. Concomitant with a transcriptional upregulation of SUMO pathway components, SUMOylation was also stimulated developmentally within the maturing endosperm. We developed proteomic methods combined with transgenic germplasm to identify maize SUMOylation targets, and have isolated a *UniformMu* insertion line that suppresses stress-induced SUMOylation to dissect the function(s) of SUMO before and during stress. Collectively, these studies define the organization of the maize SUMO system and provide a springboard for functional characterizations, especially with respect to its role(s) in stress protection and development.

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

**P81**

# Disruption of one of the two maize paralogs of AtDWF1 blocks brassinosteroid biosynthesis in *nana plant2*

(submitted by Norman Best <nbbest@purdue.edu>)
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Phytohormone regulation of plant architecture including control of plant height, branching, and flowering has been extensively studied. Yet, how the integration of concurrently acting phytohormones affects plant development is unknown. We determined that the classical dwarf mutant *nana plant2* (*na2*) is a brassinosteroid (BR) deficient dwarf caused by a loss-of-function mutation in a maize ortholog of AtDWF1. *na2* mutants are deficient in BR accumulation. They accumulate the substrate 24-methylenecholesterol, and concentrations of all measured downstream BR metabolites were diminished. BR-deficient and GA-deficient mutants exhibit changes in the persistence of reproductive organs in maize flowers and extreme dwarfism. We investigated the genetic interactions between BR and GA biosynthetic dwarf mutants *na2* and *d5*. Whereas *d5* tillers profusely and induces stamen production in the ears, *na2* retains pistils in the tassel flowers. Double mutant analyses indicated that the interaction between BR and GA influence plant growth differently depending on the developmental context. Disruptions of BR and GA biosynthesis influenced height additively. *na2* was epistatic to *d5* for tiller development but *d5* was epistatic to *na2* for pistil development in the male tassel. No interaction was observed between for anther development. In addition, we also sequenced mRNA from BR biosynthetic mutant (*na1*, *na2*, and *brd1*) seedlings. Accumulation of mRNA encoding the GA biosynthetic enzymes *ent*-kaurene synthase and *ent*-kaurene oxidase was greater in the mutants compared to WT, suggesting that GA biosynthesis is up-regulated in BR deficient mutants. Our results indicate that the interactions between BR and GA are developmentally specific. These findings suggest BR and GA interaction does not fit a single inclusive interaction scheme (or module) but more likely results from the utilization of different signal transduction pathways dependent upon the developmental context.

Funding acknowledgement: National Science Foundation (NSF)

**P82**

## Dissecting the Role of Source-Sink Cross-talk in Regulation of Senescence Using a Systems Genetics Approach

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Senescence is a highly regulated process which profoundly affects productivity of annual crops by diminishing photosynthetic assimilation. Partitioning of sugars between source and sink plays a key, yet not well-understood, role in the regulation of senescence. We are using a systems approach involving genetic, metabolic and physiological characterization of natural variation, transcriptomic analyses, and forward genetics to understand the regulation of senescence by source-sink cross-talk in maize. After screening natural genetic variation in a diversity panel and a bi-parental (IBM) population, we identified candidate genomic regions underlying the regulation of senescence. Near isogenic lines (NILs) have confirmed the effect of two of the QTL. Backcross populations involving these NILs were screened to identify crossover events that narrow down the introgressions, and these events will be used to fine-map underlying genetic elements. Metabolic and physiological analyses of diverse maize inbreds indicate that maize genotypes with the ability to effectively partition sugars to an alternative (stalk) sink maintain higher photosynthetic activity upon loss of their primary (grain) sink. We have generated leaf transcriptome of B73 inbred plants undergoing premature senescence due to absence of a grain sink using RNA-sequencing. To complement natural diversity analyses, we are also using forward genetics and have identified interesting EMS mutants with a disrupted senescence program. Together, this project will enhance our overall understanding of the regulation of senescence via source-sink cross-talk. Such knowledge will provide an opportunity to enhance carbon yield by manipulation sugar partitioning and delaying the onset of senescence.

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

**P83**

## Does hypoxia signal transduction involve targeted ROS-mediated oxidative base modification (8-oxoG) at G4 DNA elements in maize?

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Signal transduction pathways in plants are crucial for responding to environmental and endogenous stresses, such as hypoxia. A recent study implicated G-quadruplex (G4) DNA elements in the regulation of hypoxia and energy crisis pathway genes in maize (Andorf *et al*., 2014, *J Genet Genomics* **41**:627). In that study, a model was proposed involving G4 DNA elements as key components in modulating stress response signals leading to altered gene expression patterns. Whether or how G4 DNA functions to control genes in maize is unknown, despite their conspicuous presence in and around genes. In mammalian cells, hypoxia-induced ROS causes DNA damage, but is also associated with site-specific oxidative base modification to produce 8-oxoguanine, 8-oxoG ">(Al-Mehdi, *et al*., 2012, *Sci Signal*. **5**:ra47). The 8-oxoG was found to accumulate during hypoxia in the promoters of mammalian hypoxia-induced genes. Interestingly, G4s displayed a seven-fold enrichment for 8-oxoG in a recent genome-wide study (Pastukh *et al*., 2015, *Am J Physiol Lung Cell Mol Physiol*, *1*, **309**:1367). From these and related studies, M. Gillespie and colleagues (U South Alabama College of Medicine, Mobile, AL) suggest that "the BER pathway links hypoxia-induced introduction of oxidative DNA modifications in promoters of hypoxia-inducible genes to transcriptional activation" (Pastukh *et al*., 2015). To test the hypothesis that a similar phenomena may occur at maize G4s, we are investigating 8-oxoG dynamics in response to flood-induced hypoxia in maize seedlings. These studies may provide mechanistic insight for understanding how the maize genome responds to abiotic stresses such as hypoxia, with major implications for crop improvement strategies.

Funding acknowledgement: National Science Foundation (NSF)

**P84**

## Dual DNA and RNA Purification from High-Starch Maize Seed Tissue using a High-Salt SDS Extraction Method.

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Purification of large quantities of high quality DNA and RNA from maize seed tissue has proven to be difficult in comparison to leaf or seedling tissue. This difficulty is due to interactions between the starch and nucleic acids, interfering with the purification of nucleic acids without co-purifying starch. The use of high-salt concentrations disrupts interactions between the nucleic acids and starch, allowing the starch to be precipitated without co-precipitation of nucleic acids. DNA purification from high-starch tissues using high-salt concentrations in the presence of SDS has been found to co-purify RNA. This dual purification permits creating separate DNA and RNA samples from a single seed tissue sample, simplifying downstream analysis of gDNA and RNA from common starting samples. gDNA purified by this method has successfully been used for PCR and total RNA has successfully been used for reverse transcription PCR (RT-PCR) without co-amplification from gDNA.

**P85**

## Engineering amyloplast 6-phosphogluconate dehydrogenase to improve heat stability of the oxidative pentose phosphate pathway in maize seed development

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Heat stress reduces maize grain weight and quality. Starch synthesis in the endosperm is sensitive to high temperature stress and has the potential to be a limiting pathway for grain yield under heat stress. In addition to enzymes directly involved in starch biosynthesis, plastid-localized 6-phosphogluconate dehydrogenase (PGD3) is critical for starch accumulation. PGD3 is one of three enzymes in the oxidative section of the Pentose Phosphate Pathway (PPP). Maize encodes two cytosolic versions of 6-phosphogluconate dehydrogenase, PGD1 and PGD2. Homozygous double mutants of pgd1 and pgd2 UniformMu alleles show a variably expressive defective kernel phenotype along with poor plant development. Severe double mutant seeds were shown to be impacted both in starch and oil accumulation. Using the reference pgd1; pgd2 double mutant, we found that the cytosolic PGD1 and PGD2 isozymes are heat stable, while the amyloplast-localized PGD3 is heat labile under in vitro and in vivo heat stress conditions. In order to develop a heat stable 6-phosphogluconate dehydrogenase in amyloplasts, we developed constructs to fuse the waxy1 N-terminal chloroplast targeting sequence to the Pgd1 and Pgd2 open reading frames. The WX::PGD1 and WX::PGD2 fusion proteins were in vitro transcribed and translated and import into isolated pea chloroplasts indicating that the targeting sequence is functional. Transgenic maize plants were generated to express WX::PGD1 and WX::PGD2 under the 27kD zein promoter to confer endosperm specific expression. T0 transformants showed increased 6-phosphogluconate dehydrogenase enzyme activity and isozyme activity assays suggest the increase is due to higher levels of PGD1 and PGD2 isozymes. The WX::PGD2 transgene complemented the pgd3 defective kernel phenotype suggesting the fusion protein is targeted to the amyloplast. These transgenic lines are currently being tested for increased heat stability and increased yield under high temperature stress.

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

**P86**

## Essential role of Sucrose Phosphate Phosphatase1 and sucrose re-synthesis in maize grain filling and pollen tube elongation

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Sucrose has a multifaceted and central role in plant development, including in carbon metabolism and transport as well as in regulation of gene expression. Hence, the regulation of sucrose biosynthesis is a fundamentally important and surprisingly subtle problem. An intriguing question is the metabolic significance of sucrose re-synthesis from imported sugars - a process known to be active in diverse sink organs. Sucrose Phosphate Phosphatase (SPP) catalyzes the hydrolysis of sucrose-6-phosphate into sucrose - the final, irreversible step in the sucrose biosynthesis pathway. We isolated three *Mu* tagged allele of *spp1* from UniformMu population, all of which carry the transposon insertions in the first intron of the *Spp1* gene. Homozygous seeds of these *spp1* alleles showed a classic *shrunken1* kernel phenotype, indicating that *Spp1* has an essential role for normal grain filling. This result is consistent with the Shanon's hypothesis that re-synthesis of sucrose from imported sugars has an important role in grain development. Intriguingly, an additional mutant allele with a partial deletion of the *Spp1* gene (*spp1-d1*) could not be made homozygous. Genotyping individual seeds obtained by self-pollination as well as analysis of reciprocal crosses confirmed that the *spp1-d1* deletion allele is not transmitted through the male gametophyte. In vitro pollen germination test showed approximately 50% of pollens obtained from heterozygous *spp1-d1* plants elongated pollen tubes, suggesting that *Spp1* is required for pollen tube elongation. Taken together, our results revealed for the first time in plants that SPP, and by implication sucrose re-synthesis, are essential for seed filling as well as pollen function.

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

**P87**

## Evolutionarily Conserved Regulatory Mechanisms of Seed Storage Protein gene Expression in Monocot and Dicot

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Seed development of flowering plants begins with the double fertilization. In *Arabidopsis* (dicot), the embryo morphogenesis is completed by seven days after pollination and then is followed by the maturing stage, while the endosperm begins to degenerate. As a consequence the cotyledons in embryo are the main storage organ in *Arabidopsis*. In contrast, maize seed (monocot) has a persistent endosperm, which accounts for 80-90% of dry seed weight. Although the storage organs in monocot and dicot derive from the different products of double fertilization, their seed storage protein (SSP) genes are precisely regulated in temporal and spatial specificities. Moreover, the SSP gene promoters from dicot and monocot seem to share some regulatory elements. The 27-kDa γ-zein is one of the major SSPs in maize endosperm, which plays an important role in the hard endosperm modification in quality protein maize. In order to study whether there is a common regulatory system in dicot (cotyledons) and monocot (endosperm) storage organs, we constructed a maize and *Arabidopsis* transgenic system, in which the GFP is driven by the 27-kDa γ-zein promoter. As expected in maize, GFP is strongly expressed in the endosperm, while no perceivable expression was observed in the embryo. Surprisingly in *Arabidopsis*, GFP is highly and specifically expressed in cotyledons, but not clearly detected in other tissues, suggesting the existence of common regulatory mechanisms in the origin-different storage organs in monocot and dicot. Currently, we have constructed an EMS mutant library with the P27γ-GFP transgenic *Arabidopsis* and screened a number of mutants that display the altered seed phenotype and decreased GFP expression. We are now taking advantage of next-generation sequencing to map the mutant genes. This project will provide significant insights into understanding the conserved mechanisms of SSP regulation in evolution of flowering plants.

Funding acknowledgement: National Natural Science Foundation of China

**P88**

# Exploring photosynthetically relevant variation in the maize germplasm using MAGIC

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Maize germplasm contains abundant genetic variation of interest to both breeders and geneticists. However, it is not always easy to detect and capture it. We have come up with a novel forward genetic screen to facilitate the exploration of natural variation for any trait of interest. Named MAGIC - for Mutant-Assisted Gene Identification and Characterization - this screen makes use of a mutant/variant in the trait of interest as a reporter to identify trait enhancers or suppressors (modifiers). MAGIC is especially useful if a dominant or semi-dominant mutant is used as a reporter. All it takes is crossing the reporter mutant with a collection of diverse inbred lines and then evaluating the resulting F1 progenies for changes in the mutant phenotype. Curious to figure out what kind of variation might be there in the maize germplasm to impact photosynthesis, we conducted a MAGIC screen using Oil yellow-1 (Oy1) as the reporter mutant. Oy1 is a partially-dominant mutant of magnesium chelatase, an enzyme that catalyzes the first committed step to chlorophyll biosynthesis. Genetic crosses of Oy1 with various lines, including both common inbreds and NAM founders, showed that significant enhancers or suppressors of the Oy1 phenotype are present in many maize inbreds. Interestingly, B73 suppressed the phenotype of Oy1 and Mo17 enhanced it, allowing us to make use of the IBM-RILs to genetically dissect the architecture of Oy1-modifying effect. This led to the identification of a major QTL on chromosome 10, which we have dubbed moy1 (modifier of Oy1-1). While the gene underlying moy1 is being cloned and validated by targeted mutagenesis with EMS, other moy loci are being uncovered from the NAM founders, as well as from the maize association panel by combining MAGIC and GWAS.

Funding acknowledgement: National Science Foundation (NSF), USAID

**P89**

## Expression pattern of genes selected from suppression subtraction hybridization in multiple resistant and susceptible germplasm identifies potential candidate genes for resistance to aflatoxin accumulation in corn.

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Aflatoxin is toxic and most potent carcinogenic secondary metabolites produced by a fungus Aspergillus flavus which causes severe health hazard in human and livestock when consumed in small amount. Characterization of various host defense mechanism and identification of genes involved in the resistance are extremely important for the development of resistant corn germplasms. Consistent QTL with large phenotypic effect and markers associated with them are not available till date, which hinders the marker assisted breeding program. To investigate the genes induced in response to A. flavus inoculation, suppression subtraction hybridization (SSH) library has been prepared from inoculated Mp715 (resistant) and B73 (susceptible) to identify the differentially expressed genes (DEGs). Altogether 300 DEGs were identified from SSH library and their expression has been studied by reverse northern hybridization. Several genes (including transcription factors) related to stress response, signal transduction and disease resistance were identified from the study. Thirty DEGs that potentially involved in aflatoxin resistance were selected from library for reverse transcription PCR (RT-PCR) and screened among seven inbreds (Mp715, Np719, Mp420, Mp313E, Mo18W, B73 and va35) at different time point after inoculation. Most of the genes were highly expressed in resistant inbreds as compared to susceptible inbreds. Quantitative real-time PCR (qPCR) was used to further validate the results of RT-PCR showing the higher expression of pathogenesis-related protein- 4 (PR-4), PR-5, leucine rich repeat family protein among resistant germplasm most notably in Mp719. These results helped to identify the potential biological pathways and genes, and involved in resistance and provide the way for further study of host-plant resistance and host-pathogen interaction. In-silico mapping identified many genes that are located on or nearby the QTL regions responsible for aflatoxin resistance. This integrated and comprehensive approach involving gene expression and localization of genes on QTL map would be very helpful to identify the potential candidate genes in aflatoxin resistance.

**P90**

## Fast forward genetics in Setaria viridis, a model system for Panicoideae

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Setaria viridis is an emerging model system for C4 grasses, and closely related to important crops that belong to the subfamily Panicoideae. Therefore establishing a forward genetics pipeline in Setaria viridis can speed the identification and characterization of genes of interest that can be translated to closely related crops such as maize and sorghum. We have developed an NMU mutagenized population consisting of 20,000 M2 families. Screens of approximately 3,000 families identified two mutants with a sparse panicle phenotype, named as spp1-1, and spp1-2. Bulk segregant analysis (BSA) by sequencing was used to identify two nonsynonymous changes in the gene SvAux1, that likely underly the sparse panicle phenotype. Segregation analysis indicates both are recessive loss-of-function alleles and complementation tests between spp1-1 and spp1-2 are ongoing. Observation of early development with scanning electron microscopy shows that size, shape, and position of primary branches is disrupted in the mutants, but higher order branches and spikelets appear unaffected. Synteny comparisons identified a maize ortholog of SvAux1 and mutant characterizations of this gene are ongoing. In conclusion, Setaria viridis is a tractable genetic model for rapid gene candidate identification and functional characterization. We estimate that the time from mutant screens to candidate gene identification can be as short as seven months. In contrast, the same approach in maize would take at least 14 months in a greenhouse or longer in the field. The use of Setaria viridis for BSA by sequencing to map genes of interest has the advantages of time, labor and cost saving. As an alternative to mutagenesis, we have also initiated seven quantitative trait loci (QTL) mapping populations and sequenced ~450 Setaria viridis accessions to explore natural variation.

Funding acknowledgement: Department of Energy (DOE)

**P91**

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

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An important component to increasing crop productivity is improving Nitrogen Utilization Efficiency (NUtE). In maize this trait is measured as the ratio of grain yield to accumulated plant N. Enhancing NUtE offers substantial economic and environmental benefits, but little is known about the genetic mechanisms that govern NUtE within inbred or hybrid maize varieties. Previous work has utilized a genetic mapping study of a hybrid population developed from the intermated B73 X Mo17 recombinant inbred line (IBMRIL), test crossed to the Illinois High Protein 1 (IHP1) inbred line, which has altered N utilization. We identified 9 robust strong effect QTL for NUtE that range in size from 14-9030 kbp and aim to identify causal genetic variants. Subsequent fine mapping localized the largest effect QTL to a 426 kbp region on chromosome 1 containing 10 annotated genes, and narrowed two other QTL to a single gene. Publically available data for RNA expression variation among B73 and Mo17 increases confidence for a subset of potential candidate genes that likely contribute to increases in NUtE. In addition, analysis of allelic divergence between a diverse set of Stiff Stalk and Non-Stiff Stalk inbreds, which is indicative of past phenotypic selection for high grain yield, has provided another means of prioritizing the most promising candidate genes. The phenotypic contributions of candidate genes to NUtE are being evaluated through analysis of mutants recovered from reverse genetics screens and transgenic plants. Targeted genome editing experiments have also been initiated as another means to verify gene function. The results of this project will aid the development of maize hybrids that require lower nitrogen inputs and therefore would reduce costs for farmers and mitigate environmental and health effects associated with high ambient nitrogen levels.

Funding acknowledgement: National Science Foundation (NSF), Illinois Corn Growers Association

**P92**

## Formation of Protein Storage Vacuoles in Maize Starchy Endosperm with Wheat Low Molecular Weight Glutenin

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Protein storage vacuoles is one of the major protein storage structures rare in maize starchy endosperm, but prevalent in wheat. In contrary, maize stores seed storage proteins almost exclusively in protein bodies. The cereals all share related storage proteins, called prolamins, which have diverged during evolution. Two prolamins that are closely related are the wheat Low Molecular Weight (LMW) glutenins and the γ-zeins. Therefore, we asked what suborganellar structure would form if we swapped these two prolamins in maize endosperm? Interestingly, the combination of transgenic LMW-glutenin and knock-down of γ-zeins restored the formation of the electron-dense protein storage vacuoles in maize endosperm. Additionally, LMW-glutenin compensates the phenotype of γ-RNAi and partially rescues the phenotype of the mutation of floury2 due to the restoration of near normal protein bodies. Our results indicate that the presence of γ-zeins inhibits the formation of protein storage vacuoles (PSV) in the starchy endosperm and LMW-glutenin is involved in the formation of both protein bodies and protein storage vacuoles, providing us for the first time an explanation of the molecular evolution of protein body structure.

**P93**

# From Maize to Medicine: Analysis of the human ortholog of a novel RNA Binding Motif Protein involved in disruption of maize cell differentiation and proliferation

(submitted by Paige Gronevelt <jgronevelt@oakland.edu>)
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A novel RNA binding protein (GRMZM2G163247) has recently been found to be involved in post-transcriptional processes within maize. Previous studies have shown that mutations in this gene result in aberrant endosperm cell differentiation and excess proliferation in tissue culture. The human C7ORF64 gene is orthologous to GRMZM2G163247 and currently has uncharacterized function. A striking similarity exists between the phenotype observed from mutant endosperm cultures and the deregulated blood cell production that occurs in myelodysplasia and hematological malignancies. Based on this, we sought to investigate the expression of C7ORF64 in cultures of normal human bone marrow and cell lines derived from hematological malignancies. Using RT-PCR, we found that all tested human cell types (normal and malignant) express C7ORF64 transcripts. Given this observed expression, we tested the hypothesis that C7ORF64 has similar functional effects in human cells as its ortholog in maize. Previous RNAseq analysis identified a subset of potential target genes affected by mutant GRMZM2G163247 in comparison to wild-type controls. To determine whether these candidate genes were impacted by C7ORF64, human homologs were identified. These homologs included genes involved in cell cycle regulation, DNA repair, and chromatin dynamics. RT-PCR analysis of the homologous genes revealed differential expression of transcript variants in malignant cells with minimal to no alteration in normal cell populations. The similar alteration in transcript expression patterns observed, in both mutant GRMZM2G163247 and human malignant cell lines, indicates the function of this orthologous gene may be evolutionarily conserved between plants and humans. We are in the process of creating a CRISPR/Cas9-mediated C7ORF64 knockout human cell line to better define the role of this gene in post-transcriptional processes and cell function. These studies support a paradigm shift in the way we derive our understanding of human disease and that it is possible to obtain this knowledge by transcending evolutionary borders.

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**P94**

## Functional characterization of an ABA hydroxylase as a candidate gene affecting carbon isotope discrimination in maize

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Discrimination against the stable isotope $^{13}C$ is used in $C_3$ plants to select for desirable physiological traits associated with water use efficiency (WUE, the ratio of photosynthesis to transpiration). Carbon isotope discrimination ($\Delta^{13}C$) in $C_3$ plants solely depends on the intercellular partial $CO_2$ pressure inside the leaf ($c_i$), which is directly correlated to the stomatal aperture. The wider the stomates are open the higher $\Delta^{13}C$. It has been shown that the homeostasis of the phytohormone abscisic acid (ABA) is a main driver of stomatal closure, therefore providing a link between $\Delta^{13}C$, $c_i$ and ABA levels. Knowledge about these mechanisms in the $C_4$ species maize is limited, because the relationship between $\Delta^{13}C$ and $c_i$ is not known and the metabolic pathways of ABA (which genes and proteins are involved) are not well characterized or understood. In a maize introgression library we identified an ABA hydroxylase hypothesized to be involved in the degradation of ABA as a candidate gene affecting $\Delta^{13}C$. With functional and molecular approaches in Arabidopsis, yeast and maize we analyze, how this candidate gene contributes to ABA degradation, regulation of stomatal aperture and altered $\Delta^{13}C$. Our study aims at understanding the functional contribution of the identified ABA hydroxylase to $\Delta^{13}C$ and the interrelations of $\Delta^{13}C$, $c_i$ and ABA in maize in order to potentially exploit them as selection criteria for increased WUE.

Funding acknowledgement: Deutsche Forschungsgesellschaft (DFG)

**P95**

## Gene expression variation associated with meiotic recombination in the bz1/sh1 region of Zea mays

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The process of genome evolution depends upon the creation of sequence variation in an organism's genetic code. One such source of sequence variation arises as a result of the mutagenic effects of meiotic recombination. The frequency at which recombination events occur varies significantly, both within and between species. Further, recombination frequency is inconsistent for discrete regions of the same chromosome, and is partitioned into hotspots and coldspots. It has been hypothesized that recombination leads to regions of elevated mutation due to chromosomal rearrangements and inaccurate break repair, and that these regions experience rapid fixation of alternate alleles in a population. The bz1/sh1 region of Zea mays serves as an excellent model that has been utilized to understand the influence of recombination on genetic variation. Two maize inbred lines, W22 and B73, exhibit extensive sequence variation in the intergenic regions across the bz1/sh1 interval while maintaining colineartiy and sequence conservation of the resident genes. Analysis of this region uncovered signification allelic expression variation for genes adjacent to previously identified recombination hotspots from maize hybrids of various genotypes. Of particular interest is the considerable expression variation that is observed near sesquiterpene cyclase (stc1) and tac6058 (gene of unknown function) and a second upstream region containing a putative transcription factor (ptf) and apetala2 (ap2). In the present study, both hemizygotes and recombinant individuals, identified via flanking phenotypic markers, were used to delineate the association of recombination frequency with expression divergence. Breakpoints were identified via a PCR based method designed to exploit the sizeable indels unique to each parental line. Preliminary results show recombination hotspots near significantly differentially expressed genes, despite disparity in genome structure and content at these two locations. Further, the hottest spot is directly adjacent to the coldest spot, and both lie within an area lacking both TE insertions and methylation

Funding acknowledgement: National Science Foundation (NSF)

**P96**

## Genetic Control of Biochemical Defense against *Fusarium graminearum* is Revealed by Metabolomics and Quantitative Genetics

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*Fusarium graminearum* is one of the most destructive and widespread fungal pathogens attacking grain crops. In maize, it is a major concern due to mycotoxin contamination of kernels, as well as negative effects on yield from Gibberella stem rot and Gibberella seedling blight. Previous genetic mapping of *F. graminearum* resistance in maize has identified a large number of environment-dependent quantitative trait loci (QTL) with small effect sizes. In this project, we focus on a developmental stage (V3 seedlings) and a tissue type (roots) that are less commonly targeted in maize-*F. graminearum* interaction studies, investigate better-defined quantitative traits, and perform artificial inoculation experiments using diverse maize and fungal genotypes in growth chambers. In many but not all maize inbred lines, *F. graminearum* inoculation of seedling roots leads to root growth inhibition and shoot elongation. These early morphological changes are significantly correlated with later seedling lodging rate. Inbred lines B73 and Mo17, which exhibit contrasting *F. graminearum*-induced morphological changes, have significantly different root metabolite profiles. Many metabolites that are induced by *F. graminearum* infection in B73 are constitutively abundant in Mo17. Metabolite profiling, in combination with QTL mapping using a B73 x Mo17 recombinant inbred population, identified not only genomic hotspots affecting multiple root metabolites, but also specific loci controlling the constitutive abundance of *F. graminearum*-induced metabolites.

Funding acknowledgement: United States Department of Agriculture (USDA), Northeast Sustainable Agricultural Research and Education (NESARE)

**P97**

## Genetic mapping shows intraspecific variation and transgressive segregation for caterpillar-induced aphid resistance in maize

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Plants in nature have inducible defenses that sometimes lead to targeted resistance against particular herbivores, but susceptibility to others. The metabolic and genetic diversity available for maize make this a suitable system for a mechanistic study of within-species variation in such plant-mediated interactions between herbivores. Beet armyworms and corn-leaf aphids are naturally occurring maize herbivores with different feeding habits. Whereas chewing herbivore-induced methylation of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside (DIMBOA-Glc) to form 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside (HDMBOA-Glc) promotes caterpillar resistance, lower DIMBOA-Glc levels favor aphid reproduction. Thus, caterpillar-induced DIMBOA-Glc methyltransferase activity is predicted to promote aphid growth. To test this hypothesis, the impact of caterpillar feeding on aphid progeny production was assessed using seventeen genetically diverse maize inbred lines. Whereas aphid progeny production was increased by prior caterpillar feeding on lines B73, Ki11, Ki3, and Tx303, it decreased on lines Ky21, CML103, Mo18W, and W22. Genetic mapping of this trait in a population of B73 x Ky21 recombinant inbred lines identified significant quantitative trait loci on maize chromosomes 1, 7 and 10. There is transgressive segregation for aphid resistance, with the Ky21 alleles on chromosomes 1 and 7 and the B73 allele on chromosome 10 increasing aphid progeny production. The chromosome 1 QTL coincides with a cluster of three maize genes encoding benzoxazinoid O-methyltransferases that convert DIMBOA-Glc to HDMBOA-Glc. Gene expression and benzoxazinoid measurements indicate that caterpillar-induced responses in this pathway differentially affect aphid resistance in B73 and Ky21.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P98**

## Host-induced gene silencing using the ver-1 gene from *Aspergillus flavus* to reduce aflatoxin contamination in maize

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Maize (*Zea mays* L.) is one of the major crops susceptible to *Aspergillus flavus* infection and subsequent contamination with aflatoxins, the most potent naturally produced carcinogenic secondary metabolites. This pathogen has the potential to cause severe economic losses due to aflatoxin contamination in food and feed. The currently strategies to manage aflatoxin contamination, which include biocontrols and resistant cultivars, have limitations. Hence, a novel host induced gene silencing (HIGS) strategy was employed in this study. The *A. flavus* gene ver-1 encoding versicolorin, a key enzyme involved in the aflatoxin biosynthetic pathway, was selected as a possible target for suppression through HIGS. An RNAi vector containing portion of the ver-1 gene was constructed and introduced into immature B104 maize zygotic embryos through Agrobacterium transformation. Twenty-three transgenic plants from seven independent transformation events were produced. Polymerase chain reaction (PCR) analysis of the genomic DNA from leaf tissue confirmed the presence of the transgene in six of the seven events. Real-time reverse transcription PCR (qRT-PCR) analysis of RNA isolated from transgenic leaf tissues showed a high level of variation in fungal target gene expression among the transgenic leaf tissues. The differences in aflatoxin production between the control and transgenic maize kernels were analyzed using HPLC seven days after inoculation with *A. flavus* and incubation under kernel screening assay conditions. Kernels from two events out of four examined had less aflatoxin ($p=0.01$ and $p=0.08$) than control kernels. The results from these preliminary studies suggest that the ver-1 gene can be used to reduce aflatoxin contamination in maize through HIGS.

Funding acknowledgement: The Aflatoxin Mitigation Center of Excellence (AMCOE) and USDA Coperative Agreement

**P99**

## Identify Gene Regulatory Network Controlling Phenolic Biosynthesis in Maize

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Maize accumulates large numbers of phenolic compounds, including phenylpropanoids, lignin and flavonoids, which play important roles in plant growth and adaptation. Lignins are crucial for biomass production, and flavonoids are key nutraceuticals providing value to human and animal diets. The goal of this study is to identify genome-wide regulatory networks that control maize phenolic biosynthesis. We developed and released a publicly available maize TF ORFeome library consisting of 2,034 transcription factors in recombination-ready vectors. We identified genome-wide transcription start sites (TSSs) of phenolic biosynthesis genes using Cap Analysis Gene of Expression (CAGE) in roots and shoots of the two widely utilized maize inbred lines, B73 and Mo17. Based on the TSSs mapping, upstream (~1 kb) regulatory regions of 55 genes responsible for phenolic synthesis in B73 maize lines were cloned and individually integrated into the genome of yeast. These yeast integrated regulatory regions were used as baits in yeast one-hybrid (Y1H) screens to identify novel protein-DNA interactions (PDIs). So far we identified ~865 PDIs, comprising 496 TFs and 55 promoters. Selected PDIs were further validated in maize protoplasts by chromatin immunoprecipitation (ChIP) and transient activation assays. Information derived from these studies is being integrated into the GRASSIUS (grassius.org) knowledge base, contributing to develop a first map of the regulatory motifs that participate in the control of maize phenolic biosynthesis.

Funding acknowledgement: National Science Foundation (NSF)

**P100**

## Identifying conserved transcriptome responses to thiamin deficiency in maize and Arabidopsis

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Thiamin (vitamin B1), in its diphosphate form, is an essential cofactor for many enzymes in key metabolic pathways such as glycolysis, the pentose phosphate pathway, and branched chain amino acid biosynthesis. In spite of these important physiological roles, information on how thiamin affects gene expression and metabolism in plants is limited. Here, we analyzed transcriptome profiles of the maize thiamin auxotroph mutant *baldekiller1-R* (*blk1-R*) and the orthologous Arabidopsis mutant *tz*, and compared these profiles in order to identify commonalities and differences in gene expression response to thiamin deficiency. We sampled shoot apices of developing maize seedlings at the 6-7th leaf stage and 10-12th leaf stage. At the 6-7th leaf stage, mutants lack a visible phenotype, whereas narrow leaves emerge at the 10-12th leaf stage. We identified only 42 genes with significant changes in transcript level at the 6-7th leaf stage. In contrast, at the 10-12th leaf stage, 5639 genes showed significantly different transcript levels between wild type and *blk1-R*. For the Arabidopsis RNA-seq analysis, we used 17-day-old rosette leaves from plants grown on medium containing 30 nM thiamin (a concentration that results in low thiamin status). We identified 195 genes that showed significant differences between wild type and *tz* mutant. Among these 195 genes, 39 were also affected in the maize *blk1-R* mutant at the 10-12th leaf stage. These transcriptome profiles provided insights into how thiamin deficiency affects plant gene expression. Combined with metabolite profiles, this information will facilitate construction of transcriptome-based metabolic models to predict the impact of thiamin deficiency on plant metabolism.

Funding acknowledgement: National Science Foundation (NSF)

**P101**

## Inhibited sucrose transport in *Carbohydrate partitioning defective1* is associated with altered vasculature and enhanced plant defense responses

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Carbohydrate partitioning is the process by which plants transport sugars, predominantly sucrose, which is synthesized in the photosynthetic source tissues (e.g., leaves) to non-photosynthetic sink tissues (e.g., roots, flowers, fruits, and seeds). Despite the essential nature of this process little is known about its genetic basis. *Carbohydrate partitioning defective* (*cpd*) mutants are incapable of transporting sugars properly from source to sink tissues, which results in accumulation of carbohydrates in the leaves. *Cpd1* is a semi-dominant mutant whose phenotype consists of chlorosis, starch and anthocyanin accumulation in the leaves, and short stature plants. Interestingly, the *Cpd1* mutant is unique from other *cpd* mutants in that it exhibits excess callose deposition and ectopic lignin in the phloem tissue, increased aphid resistance, varying levels of caterpillar resistance, and increased benzoxazinoid levels. In order to clone the gene underlying the *Cpd1* mutation, a positional cloning strategy is being used. A polymorphic mapping population was created and bulked segregant analysis (BSA) was conducted to determine the chromosomal region containing the mutation. Fine mapping is currently in progress. Mutants such as *Cpd1* are valuable for understanding the genes functioning in whole-plant carbohydrate partitioning, as well as vein patterning and insect resistance. The knowledge gained through this project is essential to elucidate the metabolic pathways related to sugar transport as well as to guide possible genetic improvements of C4 grasses, such as maize, sweet sorghum, and sugarcane, that are key to food and fuel production.

Funding acknowledgement: National Science Foundation (NSF)

**P102**

## Integrated BSR-seq and Exome-seq pipeline to discover causal deletions in B73 maize kernel mutants

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Complete endosperm filling and vitreous texture formation are essential parts of maize grain development. The molecular genetic control of these characteristics is only partially understood. We describe a novel functional genomics platform to investigate the developmental genetics of endosperm filling and vitreous endosperm formation which combines BSR-seq (Bulked Segregant RNA-seq) and Exome-seq (exon capture sequencing) to map causative mutations and identify candidate genes within mapping intervals. Using gamma-irradiation of B73 maize, we created a population of 1788 B73 lines including 47 Mo17 x B73 F2s showing stable, segregating and viable opaque and reduced fill kernel phenotypes. As proof-of-concept, we present detailed characterization of line 937; an opaque mutant harboring a 6203-bp in-frame deletion covering six exons within the *Opaque-1* gene. In addition, we describe mutant line 146 which contains a 4.8 Kb intra-gene deletion within the *Sugary-1* gene and line 916 in which an 8.6 Kb deletion which knocks out a *cyclin* A2 gene. Therefore, this study demonstrates the utility of gamma irradiation for forward and reverse genetics in large non-dense genomes such as maize since deletions often affect single genes. Furthermore, we show how this classical but underutilized mutagenesis method becomes more applicable when combined with state-of-the-art genomics tools.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P103**

## Investigation of a QTL for herbivore-induced terpene production in maize

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Volatile terpenes are an important part of the defense against herbivores in maize seedlings. When a herbivore feeds on the plant, a complex blend of volatiles is emitted. This terpene blend attracts natural enemies of the herbivores, which reduce the herbivore damage to the maize plant.
The regulation of terpene biosynthesis is complex and follows different induction patterns. We aim to identify genes that encode components of the signaling cascade from herbivore feeding to the up-regulation of terpene production.
We utilized Nested Association Mapping to identify factors that control terpene emission. We screened the volatile profile of 5'000 recombinant inbred lines and its respective 26 parent lines for volatile emission after herbivory. Within the locations of the Quantitative Trait Loci (QTL) for terpene emission, we screened for genes that may encode either structural enzymes of terpene biosynthesis or regulatory elements.
We identified a region on chromosome 9, which controls the emission of the terpenes bergamotene, farnesene, TMTT, and nerolidol. This region does not encode enzymes of terpene biosynthesis. Also, the affected volatiles are products of different biosynthetic pathways. Candidates for regulatory genes at this QTL are a kinase, and three transcription factors. The transcript levels of these candidates are controlled by herbivory. The role of these genes in the regulation of terpene synthesis is currently being investigated.

Funding acknowledgement: DFG

**P104**

## Isolation and identification of proteins associated with paramutation of B1 in maize using a two-transgene system.

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Paramutation is the heritable change of an allele induced by another allele of the same locus. This kind of trans-homolog interaction has been characterized in several plant and animal models. However, much is still unknown about the exact mechanism by which paramutation occurs. In maize, the B1 gene encodes a transcription factor that activates anthocyanin production. When a silenced B1 gene is heterozygous with a previously active allele, the active allele is paramutated, resulting in decreased anthocyanin production. This paramutation is then stable even after multiple generations. Experiments have traditionally used forwards genetics to understand what proteins have substantial roles in the phenomenon. However, advances in mass-spectrometry based proteomics can provide data from a different perspective, directly identifying proteins involved in paramutation.
In order to isolate the proteins involved in B1 paramutation, we will employ a dual-transgene system. One transgene includes a seven-repeat sequence necessary for silencing to occur, cloned adjacent to multiple repeats of the GAL4 upstream activation sequence. The second transgene possesses the GAL4 protein translationally fused to a FLAG epitope tag. When plants transgenic for each single transgene are bred together, and the two transgenes are expressed in the same F1 plants, immunoprecipitation can be used to isolate the repeats and associated proteins from genomic DNA. After isolation, the proteins will be purified and submitted to analysis by mass spectrometry, using translated genomic data to provide a key for identifying all proteins captured.
Using this system, we hope to identify regulatory proteins involved in the establishment and maintenance of paramutation and associated gene silencing events.

Funding acknowledgement: National Science Foundation (NSF)

**P105**

## iTRAQ-based quantitative proteomic analysis reveals new metabolic pathways responding to chilling stress in maize seedlings

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Chilling stress is one of the major threats to plant growth, spatial distribution, agricultural productivity and crop of yield. In this study, we conducted an iTRAQ-based quantitative proteomics analysis to compare the abundance of proteins in maize seedlings under normal conditions and chilling stress. A total of 88 up-regulated and 77 down-regulated proteins were identified under chilling stress. This result demonstrates the remarkable metabolic flexibility of maize leaves, which may contribute to the survival of plants under chilling stress. The adaptive response of maize leaves to chilling stress might include the following aspects: (a) the induction of stress-responsive proteins; (b) the improvement of the overall ability to scavenge ROS, including detoxifying enzymes and compatible solutes; (c) the up-regulation of the expression of all protein synthesis/assembly-related proteins; and (d) posttranscriptional and posttranslational modifications. This approach identified new proteins involved in signal transduction, RNA metabolism, protein metabolism and other biological processes that were not previously known to be associated with chilling stress responses. Our results revealed complex changes at the proteomics level in maize leaves under chilling stress conditions and provided new information concerning the plant response to chilling stress. Additional studies are essential to improve the chilling stress tolerance of maize.

Funding acknowledgement: the National Transgenic Crops of New Varieties Breeding Major Project-New Germplasm Combination Breeding of Cold Tolerance Transgenic Maize (20142X0800305B)

**P106**

## Leveraging Germplasm Enhancement of Maize (GEM) resources to develop cultivars with tolerance to drought and MLND for Tanzania

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Maize is a major crop - grown in 3.3 million ha - in Tanzania providing 61% of dietary calories and >50% of utilizable protein. However, the average yield ranges only between 1.2 and 2.0 tons/ha while the yield potential is 8.0 tons/ha. The main reason is prevalence of biotic and abiotic stress factors including drought and maize lethal necrosis disease (MLND). There is need to identify new germplasm sources and tools for fast-tracking the breeding efforts as Tanzania relies heavily on conventional breeding methods. The Germplasm Enhancement of Maize (GEM) resources could be explored for new sources of tolerance. Doubled haploid (DH) GEM materials have been developed at Iowa State University (ISU) including GBS and SNP marker data for mapping studies to identify segments associated with any trait. Here, we aim to leverage GEM-DH and develop new materials, which will be screened in the US and in Tanzania. Eventually, segments conferring tolerance will be introgressed into Tanzanian elite germplasm. For drought tolerance, in addition to field evaluation of GEM-DH in Tanzania, hydroponic conditions (induced by polyethylene glycol) have also been used at per se and testcross level. Further, gene expression analysis for phytosterols and ascorbate pathways will be conducted in selected maize GEM-DH candidate lines. Also, candidate genes from maize predicted to encode regulators of sterol pathway have been transformed into model grass species (*Brachypodium*) to evaluate their roles and will be used as markers. Tangential activities will include training of Tanzanian researchers to use the hydroponic method of screening for drought tolerance in maize. For MLND tolerance, MLN and occurrence of respective virus isolates is mapped throughout Tanzania. In addition to screening GEM-DH material, resistance segments are being introgressed into elite Tanzanian germplasms from previously characterized resistance sources. Mapping additional MLND resistance genes from these sources are also underway.

Funding acknowledgement: USAID - iAGRI

**P107**

## Lignin Biosynthesis Pathway Plays Important Role in Rp1-Mediated Defense Response

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Disease resistance (R) genes encode nucleotide binding leucine-rich-repeat (NLR) proteins that confer resistance to specific pathogens. Upon pathogen recognition they trigger a defense response that usually includes a so-called hypersensitive response (HR), a rapid localized cell death at the site of pathogen infection. Intragenic recombination between two maize NLRs, Rp1-D and Rp1-dp2, resulted in the formation of a hybrid NLR, Rp1-D21, which confers an autoactive HR in the absence of pathogen infection. From a previous QTL and genome wide association study, we identified genes encoding two key enzymes in lignin biosynthesis, hydroxycinnamoyltransferase, HCT and caffeoyl CoA O-methyltransferase, CCoAOMT, adjacent to the SNPs which were highly associated with variation in the severity of Rp1-D21-induced HR. We provide evidence that the two maize HCTs and also CCoAOMT, suppress the HR conferred by Rp1-D21 in a heterologous system through physical interaction. The metabolic activities of HCT and CCoAOMT are unlikely to be necessary for their roles in suppressing HR. We show that the lignin biosynthesis pathway is activated by Rp1-D21 at both the transcriptional and metabolic levels. We also demonstrate that CCoAOMT2, HCTs and Rp1 proteins form the same complex(es). A model is derived to explain the roles of HCT and CCoAOMT in Rp1-mediated defense resistance.

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

**P108**

## Maize GCN2 phosphorylates eukaryotic translation initiation factor 2α under amino acid starvation and regulates the endosperm specific transcription factor Opaque2

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General control non-derepressible-2 (GCN2) plays an important role in cellular responses to amino acid availability as a regulatory protein kinase. It phosphorylates the α subunit of the trimeric eukaryotic translation initiation factor-2 (eIF2), which in turn decreases the general rate of protein synthesis in response to amino acid starvation. The phosphorylation of eIF2α enhances the translation of the transcription factor GCN4 by overcoming the inhibitory effect of the GCN4 upstream open reading frames (uORFs), resulting in increased expression of over 30 amino acid synthesis genes. Although the GCN2-like kinases are highly conserved among eukaryotes, there are no candidates of plant GCN4 homologues identified.
Mutator tagged GCN2 null mutants were used to characterize the GCN2 homologue in maize (Zea mays). ZmGCN2 shared sequence identity in the conserved domains with other GCN2 homologues. An increase of eIF2α phosphorylation in response to herbicide treatment that inhibited amino acid biosynthesis was only detected in wild type maize endosperms, not mutant, indicating that it was GCN2-dependent. Opaque2 (*O2*) was reported to have sequence and function similarity with GCN4, and its protein accumulation increased during induced endosperm amino acid starvation, but *O2* transcript level was unchanged. This suggested that O2 was post-transcriptionally regulated through the GCN2 kinase pathway and that O2 could be a maize GCN4 homologue. Bioinformatics approaches are being taken to identify other possible GCN2 responsive transcripts with highly conserved uORF spacing patterns similar to *O2*.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P109**

## Maize NAM founder lines exhibit diverse responses to caterpillar herbivory

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The maize (*Zea mays* ssp. *mays*) plant has to face attacks from numerous insects during its life cycle in the field; thus it needs to employ an array of defense responses to deter insect herbivory. The fall armyworm (*Spodoptera frugiperda*) (FAW) is a major caterpillar pest on maize across southeastern USA and South America. Currently, there are few studies on how the NAM founder lines respond to caterpillar herbivory; hence, this study focuses on assessing maize resistance to FAW in the founder lines and illustrating possible resistance mechanisms. Insect bioassays indicated that the constitutive defense of the 25 founder lines fall into a continuum. Inbred lines in the tropical or semitropical group possess more resistance than those in other genetic groups. After the initial assessment, six founder lines, B73 and Mp708 were selected for further study. Previous research demonstrated that Mp708 is highly resistant to FAW. A second bioassay divided these eight genotypes into five susceptible and three resistant lines. For induced defense, FAW growth inhibition divided them into highly, medially and minimally inducible genotypes. At the molecular level, ribosome-inactivating protein 2 (RIP2), a known maize herbivore defense protein was studied. A higher constitutive level of *rip2* mRNA and low constitutive amount of RIP2 protein was present in NC350, CML333, and Mp708 control plants, which partially contributed to their high constitutive defense. For susceptible genotypes, the accumulation of *rip2* mRNA and RIP2 protein only increased after FAW infestation. Il14H gave the most interesting result. RIP2 protein was not triggered by FAW infestation, which correlated well with no induction of Il14H *rip2* mRNA. The study identified the genotypes with high constitutive and induced defenses to FAW and has begun to investigate the molecular resistance mechanism. The eight selected lines exhibited diverse responses to FAW herbivory at both phenotypic and molecular levels.

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

**P110**

## Maize *Opaque10* encodes a cereal specific protein that controls protein body morphology by determining the proper distribution of zeins

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The maize protein bodies (PBs) are formed by orderly packing of different zeins. However, the exact process of such packing remained poorly understood. Maize *opaque10 (o10)* is a classic opaque endosperm mutant with misshapen PBs. In this study, *O10* was cloned by positional cloning. It encodes a novel cereal specific protein with three functional domains. The middle domain contains a seven-repeat sequence that is responsible for its dimerization. The C-terminal domain contains a transmembrane motif that is required for its ER localization and PB deposition. The N-terminal domain can interact with 19-kD, 22-kD α-zeins, and 16-kD, 50-kD γ-zeins. Cellular fractionation assay indicated that O10 is initially synthesized in the cytoplasm, then anchored to the ER, and eventually deposited into the PB. Immunolocalization of O10 in PB showed it co-localizes with 16-kD γ-zein and 22-kD α-zein, forming a ring shape structure at the interface between the α-zein-rich core and the γ-zein-rich peripheral region. Lose of O10 function disrupts such ring shaped distribution of 22-kD and 16-kD zeins, and resulted with misshapen PBs. These results demonstrated that O10, as a novel PB protein, determines the ring shaped distribution of 22-kD and 16-kD zeins, and controls PB morphology in maize endosperm.

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

**P111**

## Maize *Opaque11* encodes an endosperm specific transcription factor that regulates carbon and nitrogen metabolism and kernel development

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Maize *opaque11 (o11)* is a classic opaque endosperm mutant with small kernels. The mature seeds of *o11* have decreased starch and increased protein contents. During seed development, *o11* has much smaller starch granules and protein bodies in endosperm and deformed scutellum in embryo. The *O11* gene was cloned by map-based cloning. It encodes a transcription factor (TF) that specifically expressed in endosperm. RNA-Seq analysis for *o11* mutant kernel revealed about 1,200 differentially expressed genes (DEGs) covering a wide range of functions. Chromatin immunoprecipitation coupled to high-throughput sequencing (ChIP-Seq) analysis detected about 9,000 DNA binding sites of O11 distributed over 4,000 genes. Overlay of the RNA-Seq and ChIP-Seq results revealed 162 potential O11-modulated target genes mainly associated with carbohydrate and amino acid metabolism and freezing response. O11 also directly regulates five ESR specific genes and two important endosperm specific TFs (O2 and PBF) for zein gene regulation. These results indicated that O11 functions as a core regulator for endosperm development and storage component metabolism.

Funding acknowledgement: National Natural Sciences Foundation of China

**P112**

## Maize pathogens suppress inducible phytoalexin production to thwart innate plant immunity

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Kauralexins (KA) and zealexins (ZA) are newly described secondary metabolites in maize that serve as inducible chemical defenses against insects and pathogens. In contrast to the abundance of terpene volatiles in leaves, these non-volatile terpenoid phytoalexins are only mildly produced in response to insect herbivory; however, they are strongly induced by pathogen infection. While KAs and ZAs demonstrate antimicrobial properties, the aptitude of pathogenic fungi to manipulate their production remains unknown. We profiled phytoalexins and other common defense metabolites in nine commercial hybrid lines infected with three diverse pathogenic fungi. The necrotroph *Cochliobolus heterostrophus* induced a typical response with heightened levels of jasmonic acid, 12-oxo phytodienoic acid, salicylic acid, and phytoalexins including a novel terpenoid zealexin, designated zealexin A4. The hemi-biotroph *Colletotrichum graminicola* caused strong suppression of both kauralexins and zealexins. Interestingly, kauralexins were significantly active against *C. graminicola* as evidence by reduced growth when exposed to endogenous occurring levels of kauralexins A2/B2 in anti-fungal bioactivity assays. Similarly, *Fusarium graminearum* infection repressed zealexin A4 production in infected maize tissues, and growth of *F. graminearum* was highly inhibited by zealexin A4 *in vitro*. These data indicate that maize pathogens manipulate phytoalexin production in attempt to avoid effective innate immunity.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P113**

## Mapping the Gene Responsible for *Carbohydrate Partitioning Deffective*29

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Carbohydrate partitioning is the process of assimilating carbon into photosynthates and the allocation of carbohydrates produced in source tissues (e.g., leaves) to non-photosynthetic sink tissues (e.g., seeds, roots, stems). Plants with mutations in the genes controlling these processes are unable to effectively transport sugars throughout the plant, and are therefore deemed *carbohydrate partitioning defective* (*cpd*). Due to their inability to efficiently transport sucrose from the leaves, hyperaccumulation of starch is observed. In association with this overabundance of starch, a number of notable phenotypes are used to distinguish these individuals in the field, such as the accumulation of anthocyanin in leaves, chlorosis of the leaves, and stunted growth. The aim of this study is to identify and characterize the *Cpd29* mutation, which exhibits a dominant inheritance pattern. Upon introgression into the Mo17 genetic background, the mutant phenotype was more severe than in the B73 background; therefore, the Mo17 population was primarily used for genetic mapping studies. The gene responsible for the *Cpd*29 mutation was determined to be on chromosome 10 by Bulk Segregant Analysis (BSA) mapping. Fine mapping using polymorphic PCR-based markers narrowed down the gene's location. The region has been refined to a small region and potential candidate genes are being sequenced. Once the causative gene responsible for the *Cpd*29 mutation is identified, we will determine the specific role it plays in carbohydrate transport and allocation in maize. With the identification and characterization of this gene, we will further expand our understanding of carbohydrate partitioning, and apply this knowledge to increase crop yield and food security.

Funding acknowledgement: National Science Foundation (NSF)

**P114**

## Maysin biosynthesis in maize: identification and characterization of genes related to *salmon silks* mutants

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Maysin is a C-glycosyl flavone that presents anti-feedant effects on corn earworm (*Helicoverpa zea*, CEW), an insect pest that affects maize yield each growing season. Maysin accumulates mainly in silks, and is also found to a lesser extent in pericarps, the outermost layer of the kernel. *Salmon silks* (*sm*) mutants display a salmon silk phenotype that also involves browning color after cutting and lack of maysin accumulation. Previous studies showed *sm* phenotypes to depend on *P1* (encoding an R2R3-MYB transcription factor). Although mutant analyses suggested that *SM2* has rhamnosyl transferase activity and *SM1* is involved in the last dehydration step of maysin formation, the molecular identity of the *sm* loci remains unknown. Here, we report progress on the molecular characterization of SM1 and SM2, involved in the last steps of maysin biosynthesis. Because P1 is epistatic to *SM1* and *SM2*, mRNA levels in P1-*rr* and P1-*ww* pericarps and silks were compared. Candidate genes for *SM1* and *SM2* were identified from RNA-Seq analysis by applying a discrete set of criteria. Candidate genes were then confirmed using a combination of approaches that include a novel metabolic complementation assay in maize protoplasts. We will present the molecular characterization and enzymatic activities of SM1 and SM2.

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**P115**

## Metabolic and signaling implications of the *Ndpk1* gene in maize

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Nucleoside diphosphate kinases (NDPKs) are multifunctional enzymes that maintain balanced ratios of ATP/ADP and other nucleoside triphosphates (e.g. GTP/GDP, UTP/UDP). In addition to their role in energy balance, NDPKs participate in metabolic and signaling pathways including carbon partitioning, stress tolerance, and transcriptional modulation. We have initiated four approaches to determine the roles of NDPKs in grain species using a maize model. First, analysis of the maize genome showed an NDPK gene family with nine members that encode NDPK-domain-containing proteins. These proteins cluster into four clades based on their predicted sub-cellular localization. Second, we identified maize mutants with dysfunctional expression of an *Ndpk1* gene. Their characterization is underway. Third, to determine probable sub-cellular sites of action, we transiently expressed GFP-NDPK1 in tobacco cells, and found a predominantly cytoplasmic locale in this system. Fourth, we hypothesized that *ZmNdpk1* would be sugar and oxygen responsive. This was based on previous data indicating up-regulation of cytoplasmic *Ndpk1* mRNA in seedlings growing under hypoxia, and localization of *Ndpk1* mRNAs and protein in tissues that are hypoxic and or undergoing rapid cell division (eg. potato root tips and maize endosperm). To test responsiveness, excised seedling root tips were cultured under either aerobic (20% $O_2$) or hypoxic (0.2% $O_2$) conditions with either abundant glucose (2%) or limited glucose (0.2%). After 24h, levels of *ZmNdpk1* mRNA rose in response to both hypoxia and glucose. The induction was maximal at 6h and remained stable for at least 22h. The limited effects of non-metabolizable glucose analogs (3-O-Methyl glucose and 2-deoxyglucose) indicated that metabolism, and not osmotic status, was responsible for changes in *ZmNdpk1* mRNAs. Results are consistent with proposed roles of NDPK1 in sugar metabolism of developing tissues that have high energy demands and also with proposed roles in energy metabolism under stress conditions.

Funding acknowledgement: National Science Foundation (NSF)

**P116**

# Metabolic network assisted GWAS in dry seeds of Arabidopsis and maize

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Seeds are an important source of proteins for both humans and livestock. However, major staple crop seeds, such as maize and rice are deficient in several essential amino acid (EAA) which humans can only obtain from their diet. The lack of EAA in humans diet can lead to malnutrition syndrome but despite numerous extensive efforts, only few success are known to improve EAA composition without severe yield and growth penalties. This is mainly attributes to the lack of fundamental understanding of the seed amino acids homeostasis regulation mechanisms. Recent studies have provided compelling evidence that genomics-enabled approaches such as genome-wide association studies (GWAS) can bridge this gap by unraveling genes associated with seed amino acid content. Nevertheless, GWAS is often limited and inefficient in uncovering the full genetic architecture of complex traits such as seed amino acid compositions. Our study suggests that in tightly correlated metabolic networks such as amino acid, using a combination of metabolic correlation-based network analysis with GWAS can help uncover additional genes that underlie the genetic architecture of these traits and their metabolic interactions. Testing this approach using free amino acids levels from dry *Arabidopsis* and maize seed across a characterized association panels led to unraveling additional QTLs as compared to GWAS performed only with absolute levels of free amino acids. Nevertheless, comparing the genetic basis of similar metabolic modules between maize and *Arabidopsis* free amino acid suggests that similar metabolic interactions might be regulated differently in these two systems.

Funding acknowledgement: National Science Foundation (NSF)

**P117**

## Metabolomic characterization of extracellular surface lipids throughout silk development in both inbred and hybrid genotypes of maize

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The maize silk cuticle provides a primary layer of defense against abiotic and biotic stresses during the critical period of pollen reception. Primary components of the cuticle are extracellular lipids that are infused within and laid upon the cutin matrix. These surface lipids (>100 individual metabolites) are hydrophobic and include long-chain fatty acids, hydrocarbons, alcohols, and aldehydes of 16-35 carbons in length. Previous work in inbreds B73 and Mo17 has shown that accumulation and composition varies between silks that have emerged from the husk leaves into the external environment, as compared to silks that are still encased by husk leaves. Moreover, hydrocarbons were shown to accumulate to three-fold higher levels in emerged silks from B73 compared to Mo17. Recently, we have implemented a lipid extraction method that allows us to simultaneously quantify polar and non-polar surface lipids in a single extract. We applied this method to silks from four genotypes (B73, Mo17 and their reciprocal hybrids), which were cut into five sections along the proximodistal gradient of silk development and emergence from husk leaves. Surface lipid extracts were characterized via gas chromatography-mass spectrometry (GC-MS) and subsequently analyzed using analysis of variance. To date, we have demonstrated that surface lipid accumulation patterns and composition differ among genotypes and along the lengths of the silks. For example, in comparing B73 and Mo17 we find that B73 silks have 1.4- to 2.3-fold higher accumulation of hydrocarbon end-point metabolites along the proximodistal gradient. Interestingly, the Mo17 metabolome is enriched 2- to 3.5-fold in relative fatty acid abundance (i.e., precursor metabolites) as compared to B73. These metabolomic data sets are currently being used to model the metabolic network for surface lipid accumulation. Additionally, transcriptomes of these silk samples are being sequenced to permit candidate gene identification and modeling of gene expression-metabolite accumulation dynamics.

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

**P118**

## Molecular characterization of the jasmonate receptor CORONATINE INSENSITIVE 1 in panicoid grasses

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Jasmonates (JA) are lipid-derived phytohormones that regulate a broad range of responses from growth and development to defense against biotic and abiotic stresses. The JA signaling pathway has been extensively studied in *Arabidopsis thaliana*, but differences in JA responses between monocots and dicots have been reported. In maize, mutants in the JA biosynthetic pathway revealed unique roles in sex determination and ear shoot elongation. In addition, grass systems including maize, rice, *Brachypodium distachyon* and *Setaria viridis* all have multiple copies of the JA co-receptor *CORANATINE INSENSITIVE 1* (*COI1*) while *Arabidopsis thaliana* and other dicots only have one. This suggests a more complex JA signaling network in grasses and unique roles of JA to be explored. COI1 and JASMONATE ZIM DOMAIN (JAZ) proteins form a complex in the presence of bioactive JA to trigger downstream JA responses. Here, we show that different COIs from *Setaria viridis* have diverse interaction patterns with JAZs in the presence of bioactive JA, supporting the hypothesis of a more complex signaling network in grasses through expanded COI-JAZ interactions. *coi* mutants in *Setaria viridis* were generated in our studies through CRISPR-Cas9-based genome editing. Multiple *coi* alleles were also generated in maize through *Ac*/*Ds* tagging to functionally characterize *coi* function. Outcomes from this research will reveal the role of multiple *COI*s in grasses, and provide insights into how grasses integrate developmental and environmental signals to regulate plant growth.

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

**P119**

## Molecular mapping of perennial genes in Zea L.

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A hybrid between Zea diploperennis and maize variety Rhee Flint was made to study perennialism in Zea. Here we report characterization of the hybrid-derivatives with single-sequence-repeats (SSR) and genotype-by-sequencing (GBS). The 384 SSR primers in the Sigma Maize SSR Primer Set were used to screen the parents for polymorphism. The F2 and some F3 families were screened with the identified polymorphic SSR markers. GBS data analysis of the F2 and F3 families is also in process. The results of these analyses will be presented in the poster.

Funding acknowledgement: SDSU Agricultuaral Experiment Station

**P120**

## Next-generation EMS mutagenesis increases mutation density and accelerates forward genetics in maize

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Seed mutagenesis in maize, where independent cellular lineages produce tassel and ear inflorescences, results in heterozygous M2 and slows the recovery of homozygous mutants by one generation. The easy crossing and high fecundity of maize enables EMS mutagenesis of pollen to generate populations of independently mutagenized heterozygous M1 progeny with up to one loss of function mutation per gene per ~1000 M1 plants. To overcome the limitation of low mutation frequency, we recurrently mutagenized a maize pedigree to accumulate many-fold more independent mutations per plant. We call this iterative mutagenesis approach next generation EMS mutagenesis (NextGEM). Independently derived mutations are carried as heterozygotes at every generation. Three cycles of mutagenesis produced healthy fertile progeny. NextGEM populations display dominant mutations, are selfed to recover homozygous mutants, or crossed to mutants or inbreds to conduct targeted non-complementation screens or recover an allelic series. The seeds produced by each healthy recurrently mutagenized maize plant produced an excess of seed. Thus, NextGEM populations are not one-use materials. Re-planting for additional screens, shipping of material to collaborators, or reuse for targeted mutagenesis with different loci are all possible. Crossing to existing populations allow non-complementation screening without the need for EMS application at the time of crossing. These populations carry heterozygous induced alleles and F1 progeny from crosses to mutants or inbreds carrying QTL of interest as pollen parents result in ears segregating for mutations of interest. This permits repeated family-wise observation of subtle phenotypic impacts when planted ear-to-plot and immediate linkage-mapping-by-sequencing to discover of causative polymorphisms. Thus, next generation genetic screens can take advantage of mutation discovery by whole-genome resequencing and speed forward-genetics. We are more than two generations into B73, Oh43, and Mo17 and have begun mutagenizing all of the NAM founders to permit the maize community to test QTL candidates.

Funding acknowledgement: National Science Foundation (NSF)

**P121**

## Nonsyntenic genes drive tissue-specific dynamics of differential, nonadditive and allelic expression patterns in heterotic maize hybrids

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Maize (*Zea mays* L.) exhibits an exceptional degree of structural genomic diversity. We subjected the meristematic zone, the elongation zone and cortex and stele of the differentiation zone of maize primary roots to RNA-seq to explore how the genomic divergence of the maize inbred lines B73 and Mo17 and their reciprocal $F_1$-hybrids is reflected in their transcriptomic landscapes. Based on a linear mixed model, genes were classified as differentially expressed, nonadditively expressed or showing unexpected allelic expression ratios. Differentially expressed genes were identified based on pairwise comparisons of the four genotypes. Moreover, genes whose expression in the hybrids was different from the average value of the parents were classified as nonadditively expressed. Finally, genes showing unexpected allelic ratios in hybrids significantly deviated from the expression ratios of the parental alleles. The number of differentially expressed genes between the two parental inbred lines was in all tissues higher than in the four pairwise comparisons of an inbred line with a hybrid. No differentially expressed genes were detected between reciprocal hybrids, which share the same nuclear genome. Substantial tissue-specific dynamics was observed for differential, nonadditive and allele-specific gene expression patterns in hybrids. Remarkably, nonsyntenic genes which lack syntenic orthologs in other grass species were significantly overrepresented in all these gene expression patterns. Nonsyntenic genes likely evolved after the last whole genome duplication of a maize progenitor by single gene duplications and are therefore evolutionary younger than syntenic genes. The genotype-specific expression plasticity of hundreds of nonsyntenic genes might facilitate the developmental adaptation of maize hybrids to fluctuating environments and might thus be one factor contributing to the superior performance of maize hybrids compared to their parental inbred lines.

Funding acknowledgement: Deutsche Forschungsgemeinschaft (DFG)

**P122**

## Opaque2 and Prolamin-Box Binding Factor also control kernel yield

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Maize endosperm-specific transcription factors Opaque2 (O2) and Prolamin-Box Binding Factor (PBF) belong to the bZIP and DOF family, respectively. It is already known that they regulate the expression of zein storage protein genes. We now found that O2 and PBF also control yield traits. The 1,000-Kernel (KW) and Test Weight (TW) of an *o2* variant are reduced by 20% and 13% compared to normal phenotype (WT), respectively. In addition, the KW and TW are positively correlated with the dosage of O2 in the reciprocal crosses of WT and *o2*. Although *PbfRNAi* by itself has less adverse effects than *o2*, the combination of *o2* and *PbfRNAi* has a KW and TW 43% and 23% lower than WT, respectively. This weight loss in *o2*;*PbfRNAi* correlates with a decrease of 25% starch. RNA-seq analysis of normal and mutant phenotypes reveals that expression of *PPDK1* and *PPDK2* (pyruvate orthophosphate dikinases) and *Dull1* (starch synthase III) is further reduced in the double than the single mutants. Reduction of transcript levels results in lower amounts of enzymes as well. Interestingly, the promoters of these genes contain a P and O2 box and can be synergistically trans-activated by PBF and O2. SSI (starch synthase I, the main starch synthase for amylopectin) is not directly regulated by PBF and O2, but its protein level is dramatically decreased in *o2* and further decreased in the double mutant. Previous research indicated that PPDK and SSIII are the critical components in the starch biosynthetic enzyme complexes. We propose that the down-regulation of PPDK and SSIII by *o2* and *PbfRNAi* in turn causes the decreased accumulation of SSI and consequently leads to the reduction of starch synthesis and kernel weight.

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**P123**

## Optimizing Tissue Culture Parameters for Callus Induction and Regeneration of Transgenic Sorghum Lines.

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Sorghum [Sorghum bicolor (L.) Moench] is a C4 grass of African origin. It belongs to Poaceae family and is widely cultivated in diverse climates around the world for food and forage. Sorghum is one of the most important cereals in the world after rice, maize, wheat and barley. Its high genetic diversity can be utilized to improve economically important traits to meet the challenge of climate change and ever increasing food demands across globe. It has significant genetic homology with sugarcane and maize and is an attractive candidate for energy crop due to its high biomass, yield and sugar content. It is highly stress tolerant and has high water use efficiency due to its deep root system and reduced transpiration rate thus can grow on marginal soil with low nutrient and water inputs.
Candidate genes related to economically important traits like height, biomass, maturity, tiller number, kernel weight, saccharification has been mapped by QTL mapping and association mapping using SNP and SSR markers. Mini-core collection developed at International crop research institute for semi arid tropics were used for the mapping purpose. The functional validation of the genes remains a challenge due to recalcitrant nature of sorghum genetic transformation. However, it is achievable by both biolistic (Casas et al. 1993) and Agrobacterium (Zhao et al. 2000) methods. We are using Agrobacterium mediated transformation protocols to optimize our tissue culture media for callus formation and regeneration of plants. Several explants like immature embryo, seeds, apical meristem, and leaf were used. several genotypes are tested along with different media and hormone concentration. MS based media with 2 mg per L 2,4-D and 0.2 mg per L kinetin have shown better results in callus formation. However it is too early to confirm the right media and genotype combination as several are on test.

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**P124**

## Overexpression of rice Phosphorus Starvation Tolerance 1 gene and its sorghum and maize homologs in transgenic tobacco

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Low phosphorus (P) availability in soil is a major constraint for crop production in tropical regions. Phosphorus-Starvation Tolerance1 (OsPstol1) is a protein kinase that enhances root surface, P acquisition and grain yield in rice under P deficiency. Sorghum homologs of OsPstol1 were identified by association mapping in two sorghum association panels phenotyped for P uptake, root system morphology and architecture in hydroponics and grain yield and biomass accumulation under low-P conditions, in Brazil and/or in Mali. Maize and sorghum candidate genes co-localized with quantitative trait loci (QTL) for traits underlying root morphology and dry weight accumulation under low P via QTL mapping. In order to validate the function of these genes, rice OsPstol1 (control) and its maize (ZmPSTOL3.06, ZmPSTOL8.02 and ZmPSTOL8.05_1) and sorghum (Sb07g002840, Sb03g031690 and Sb03g006765) homologs were cloned downstream of ubiquitin promoter in pMCG1005 vector, using bar gene as a selective marker. Tobacco Petit havana plants were genetically transformed via Agrobacterium tumefaciens EHA101 strain and regenerated from selected callus in shooting and rooting medium supplemented with 100 mg/ml of Tioxin and 1 mg/L of Phosphinothricin. PCR with gene specific (~700 bp) and bar (~400 bp) primers confirmed the presence of Pstol1 genes in tobacco plants. Most plants presented one copy number and overexpressed the transgene correctly. Moreover, the overexpression of Pstol1 genes significantly enhanced root surface area under low P. Currently these transgenic tobacco plants harboring the seven different genetic cassettes are being tested the enhancement of P acquisition and grain yield under low P conditions.

Funding acknowledgement: Embrapa, Fapemig, CNPq and GCP.

**P125**

## Phosphorus remobilization during maize leaf senescence

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Leaf senescence is an important nutrient recycling process. The onset and progression of senescence is controlled by both developmental and environmental factors. Phosphorus (P) is an essential but limiting macronutrient for plant growth and development. In an annual crop such as maize, efficient P remobilization during senescence is important to avoid loss of this valuable resource to non-productive tissues.
Plant purple acid phosphatases (PAPs) liberate inorganic P from P locked in organic compounds, playing an important role in promoting P availability and mobility, both in the soil, when secreted, or within the body of the plant, when acting intracellularly. We have identified 33 putative PAP encoding genes in the maize (B73) reference genome and present analysis of Pap transcript accumulation during induced leaf senescence in two inbred backgrounds and in the phosphorus transport/signalling mutant *Zmpho1;2a-m1::Ac*.

Funding acknowledgement: Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO)-Unidad de Genómica Avanzada (UGA), Centro de Investigación y de Estudios Avanzados del I.P.N. (CINVESTAV), Consejo Nacional de Ciencia y Tecnología (CONACyT).

**P126**

## PLF, a new DOF transcription factor regulates the 15-kDa β-zein gene expression in maize endosperm

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In maize (*Zea mays* )endosperm, the major storage proteins are zeins, which are divided into four classes, i.e., α (19- and 22-kDa), γ (50-, 27-and 16-kDa), β (15-kDa) and δ (18- and 10-kDa). The endosperm-specific transcription factor PBF belonging to the plant specific Dof family recognizes a 7-bp sequence element (5'-TGTAAAG-3') called the P box, which is conserved in most of zein gene promoters. Knock-down of *Pbf* with RNAi resulted in significantly reduced expression of 27-kDa γ- and 22-kDa α-zein genes. Although 15-kDa β-zein promoter contains two P boxes, its expression is not visibly affected in the *PbfRNAi* mutant, indicating another Dof TF might be involved in its regulation. Bioinformatic analysis has identified 46 Dof members in maize B73 genome, among which a second Dof gene was found to exhibit a similar expression pattern as *Pbf* after 10 DAP, and therefore it might be the candidate TF for regulating the 15-kDa β-zein gene. This new Dof gene is temporarily designated as *Plf (Pbf-like factor)*. RNA in situ experiment showed that *Plf* is specifically expressed in the starchy endosperm cells. EMSA and tobacco transient expression assays demonstrated that PLF and PBF can both recognize the two P boxes in the 15-kDa β-zein promoter and activate 15-kDa β-zein expression through interaction with O2, indicating that the two DOF TFs have redundant function in regulating 15-kDa β-zein gene expression. Unlike *Pbf*, *Plf* is also highly expressed before 10 DAP, suggesting that PLF may have functions involved in an early stage of endosperm development. We have generated three independent *Plf-Ds* mutant alleles, in which the *Ds* is inserted in the second exon at different sites. We have also taken advantage of CRISPR/Cas9, creating a number of *Plf* null mutants. The further study of PLF function is actively ongoing.

Funding acknowledgement: National Natural Science Foundation of China

**P127**

## Producing Reporter Gene Constructs for Investigating the Role of G-Quadruplex (G4) DNA elements in Gene Regulation

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Genes are known to be regulated by transcription factors that bind to specific cis-acting regulatory elements in and around the genes. Individual genes typically have unique combinations of cis-elements that collectively govern their expression patterns. Co-regulated genes often have nearby copies of the same element. G-quadruplex (G4) DNA is a widely distributed class of cis elements, prevalent in the genomes of plants, animals and bacteria, with numerous genetic functions. G4 DNA refers to small, non-duplex, 4-stranded structures that fold up within or across strands of DNA. These structures can be predicted from sequence motifs recognized computationally in genomic sequence. G4 DNA is implicated in human cancer-related gene regulation, but relatively little is known about plant G4s. Andorf et al., (*J Genet Genomics* 41:627-647) recently identified thousands of G4 motifs in stress-response genes of maize, a model genetic plant and major world crop species. We have begun to make reporter gene constructs with and without maize G4s in order to further explore the functionality of maize G4 elements. For these experiments, we selected G4 motifs from several important stress responsive genes, including *hexokinase4* (*hex4*), *shrunken1* (*sh1, sucrose synthase*) and *hypoxia-inducible related to AP2-2* (*hrap2*, a transcription factor). An expression vector, pcDNA3.1, with a lacZ reporter gene will be used to assay the effects of maize G-4 elements in the antisense 5' UTR position in *E. coli*. These constructs will be first tested in bacteria and then in eukaryotic cells, including yeast, plants and animals. Understanding how maize G4s work could shed light on how plant genes respond to stress, with implications for crop improvement strategies.

Funding acknowledgement: National Science Foundation (NSF), Women in Math Science and Engineering (WIMSE at FSU)

**P128**

## QTL regulating meiotic recombination rates

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Understanding the regulation of meiotic recombination would help breeders control how many crossover events will occur in their breeding lines and predict where the crossover events are more likely to occur. Studies in our lab have uncovered that among maize inbred lines there is a wide range of recombination rates, from about 11 crossovers per meiosis to about 19 crossovers per meiosis. However, while these differences have been documented, factors regulating recombination rate are not known. In this project, we used a QTL mapping approach to identify regions of the genome that are likely to contribute to the observed differences in recombination rates between maize inbred lines. We used genotypic data from the maize Nested Association Mapping (NAM) Recombinant Inbred Line (RIL) populations to deduce the number of crossovers accumulated in each RIL. We identified 10 unique QTL regions in eight RIL populations, located in chromosomes 1, 5, 6, and 8. We confirmed that all QTLs act in trans by removing crossovers in the QTL regions and performing the analyses again keeping all other parameters constant. We analyzed the QTL regions to identify potential candidate genes. We currently are conducting experiments to determine if the candidate genes are indeed responsible for the recombination rate differences.

Funding acknowledgement: National Science Foundation (NSF)

**P129**

## Root morphology and phosphate homeostasis in maize: the role of the ZmMed12a and ZmMed12b genes

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Mediator is a muiltiprotein complex highly conserved in all eukaryontes, functions as either a transcriptional activator or repressor. It is organized into head, middle and tail modules, as well as an additional detachable kinase module, known as the CDK8 module. MED12 is a subunit of the CDK8 module of the Mediator complex. Some mediator subunits have been found to regulate different physiological process, include the response to abiotic and biotic stress.
Recently, in our lab, it was found that loss of MED12 affects root growth and root architecture in Arabidopsis. Also miRNAs that response to phosphate starvation are derepressed in Arabidopsis med12 mutants. Maize has two ZmMED12 genes, both of which are expressed in leaves and roots. In this study, we measure root architectural traits to characterize the root system architecture and the phosphate response of maize med12 mutants. Preliminary results show that ZmMed12 mutants present some root traits associated with adaptation to low phosphorus such shallower roots and higher acid phosphatase activity. In addition ZmMed12a mutant produce more root biomass.

Funding acknowledgement: CONACYT

**P130**

## Screening for opaque2 modifiers and genes involved in kernel development by sequencing opaque revertants created by γ-irradiation mutagenesis of Quality Protein Maize

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Quality Protein Maize (QPM) is a hard kernel variety, developed from the soft, starchy kernel and high lysine opaque2 mutant by selecting opaque 2 genetic modifiers. By γ-irradiation mutagenesis of a QPM variety, K0326Y (G10, vitreous), some opaque revertants were created and preliminarily characterized. Zein protein analysis of these opaque mutants showed several different phenotypic classes affecting zein composition, including no apparent change, general reduction in zeins, and the loss of a zein. Amino acids analysis revealed that the opaque mutants have a varying degree enhancement in lysine level compared with QPM. Exon-seq analysis revealed that four mutants contain a large deletion region or single gene mutation. Further functional validation of the candidate genes is ongoing for identifying opaque2 modifier genes and general seed development genes altering the kernel phenotype of QPM.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P131**

## *Starchy endosperm cell factor 1 (Sec1)* is a new imprinted transcription factor in maize endosperm

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In *Zea mays*, endosperm is a terminal organ that is differentiated into four cell types, i.e. aleurone layer, starchy endosperm cells, transfer cell layers and embryo surrounding cells. Starchy endosperm is the most prominent part in seed, fulfilling the main role of synthesis and storage of starch and protein. Our project is to identify the endosperm-specific transcription factors and study their biological functions in the endosperm development and synthesis of storage products. We have identified a new factor, *Starchy endosperm cell factor 1 (Sec1)*, which is specifically expressed in the endosperm. Its expression could be detected by RT-PCR as early as 6 DAP and it reached the peak at 8 DAP. Sec1 was shown to localize into the nucleus, consistent with the prediction as a transcription factor. RNA in situ hybridization showed that *Sec1* is expressed across the whole endosperm at 6 DAP, but its expression is only restricted to the starchy endosperm cells after the completion of endosperm differentiation. We sequenced *Sec1* gene from more than 100 inbred lines and found *Sec1* is highly conserved and only a few inbred lines bear a couple of SNPs. We made the reciprocal crosses between inbred lines A619 and Fangyin, which have a restriction enzyme polymorphism in the coding sequence. It revealed by enzyme digestion and cDNA sequencing that the maternal allele is much more preferentially expressed in the 18-DAP endosperm cells compared to the paternal allele, resulting in the ratio of the female and male alleles significantly higher than the expectation value. Comparison of the methylation status by a methylation sensitive enzyme demonstrated that the male allele promoter is more highly methylated, indicating that *Sec1* is subject to the imprinted regulation. Now we are taking advantage of CRISPR/Cas9 to create null mutants to study its biological function.

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

**P132**

## Temperature, humidity and genotype each contribute to rates of water loss through the cuticle of maize silks: Are extracellular lipid metabolites implicated?

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The cuticle is the primary protective barrier between aerial plant tissues and the surrounding environment. Extracellular surface lipids both coat and permeate the cuticle, thereby providing a hydrophobic layer that limits transpiration and repels external water. Stigmatic maize silks are composed of ~90% water, and accumulate unusually high levels of extracellular, straight-chain hydrocarbons ranging in length from 19 to 35 carbons. The extracellular lipid metabolome on silks may have a unique set of compositional requirements based on the metabolome's function as a water barrier and the silks' function to capture and germinate airborne pollen during the critical but short period of pollination. Our group has demonstrated via metabolomic profiling that the extracellular lipid metabolomes on maize silks respond to environmental conditions, have a genetic basis, and vary along the proximodistal gradient of silk development. To investigate the protective capacity of extracellular lipids against water loss, we have implemented an experimental system to evaluate the effects of both temperature and humidity on rates of water loss from excised silks. Four IBM recombinant inbred lines and the B73 and Mo17 parental lines were selected for this analysis based on their diverse extracellular lipid metabolomes. Metabolomic profiling results were collected in tandem with water loss data from harvested silks. Regression modeling demonstrates that there are significant relationships among environmental conditions, genotype, and rates of silk water loss. For example, rates of water loss from B73 silks that had emerged from the husk leaves were lower relative to those from Mo17. Moreover, we observe a ~2-fold increase in total extracellular lipid accumulation on B73 as compared to Mo17 silks. We are currently assessing the potential correlations between levels of extracellular lipid metabolites and rates of silk water loss across temperature and humidity treatments and among genotypes.

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

**P133**

## Test of *Starch branching enzyme 1 (Sbe1)* as the source a of high amylose QTL

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In a previous study we found a quantitative trait locus (QTL) on the short arm of chromosome 5 (5S) for endosperm amylose content. In the original study, both parental lines, H99ae and GEMS-0067, were homozygous recessive for amylose extender 1 (ae1) and the polymorphism responsible for this QTL was additive (semi-dominant). Located within the QTL interval is starch branching enzyme 1 (sbe1), which makes it a candidate gene. There is a null allele of sbe1 (sbe1-Mu) that acts as a simple recessive against a functional Sbe1 allele. Interestingly, the homozygote actually decreases amylose it in ae1 background. If sbe1 is the source of the QTL, then Sbe1-H (allele associated lower amylose) and Sbe1-G (allele associated with higher amylose) will act in an additive fashion relative to each other, but each should be dominant to sbe1-Mu. If the source of the QTL is some unknown locus (unk) that is tightly linked to sbe1, then we not only expect that unk-H and unk-G will be additive with each other, but one of the alleles, most likely unk-G, will be additive with the unk allele linked to sbe1-Mu. The unk linked to sbe1-Mu is expected to be similar to unk-H because both originated from Midwestern dents, all of which have similar amylose content in an ae1 background. We have developed markers that allow us to determine the genotypes of individual kernels.

Funding acknowledgement: Department of Biology and Microbiology and SDSU Experimental Station

**P134**

## The *bif173* gene controls maize inflorescence development

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Axillary meristems are groups of undifferentiated plant stem cells that give rise to all branches and flowers, and hence are important for maize reproduction. *barren inflorescence (bif)* mutants are a class of mutants defective in axillary meristem development, and characterized by fewer flowers and branches in both tassels and ears. Several of the *bif* mutants cloned thus far have been shown to be impaired in the regulation of auxin function (biosynthesis, transport or signaling).
*bif173* is a new EMS generated mutant identified in a screen for defective axillary meristem development in inflorescences. *bif173* is caused by a single recessive mutation and is characterized by the reduction in the number of spikelets and branches in tassel and ears, a feature reminiscent of other bif mutants affecting auxin function. Genetic analysis revealed a strong synergistic interaction between *bif173* and *Bif1* and *Bif4*, two recently identified auxin signaling mutants, and suggests that *BIF173* is also involved in auxin biology. in situ hybridizations of *bif173* mutant inflorescences using different early expressed genes indicate that *BIF173* functions in the early steps of axillary meristem initiation. Interestingly, the *bif173* phenotype is more severe at high temperature, suggesting that *bif173* is a temperature sensitive mutant.
To identify the *BIF173* gene, we first followed a traditional map-based cloning approach and mapped *bif173* to a small 1.2 Mb region on chromosome 8. We subsequently performed a Bulked Segregant Analysis RNA-seq approach and identified a gene with a missense mutation in a highly conserved domain. To verify that our candidate gene is the cause of the *bif173* phenotype, we are following several complementary approaches, such as an EMS mutagenesis screening for generating new *bif173* alleles, transgene complementation, and utilizing the *CRISPR/Cas9* system to generate new lesions in our candidate gene.

Funding acknowledgement: National Science Foundation (NSF)

**P135**

## The characterization of putative DUF26 domain receptor-like kinase in maize

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Receptor-like kinases (RLKs) are a large set of trans-membrane proteins that are becoming of greater interest in the scientific community due to their important role in intercellular signaling. RNA sequence analysis of maize NAM line B73 conducted by Post Doc. Vered Tzin determined that a specific RLK, a putative DUF-26 domain receptor-like kinase, is highly induced upon caterpillar feeding. Using both *Ds* and *Illumina-Mu* knockout lines for this gene, three maize assays were conducted to help begin gene characterization. The RLK gene of interest was first over expressed through Gateway cloning techniques and coupled with a green fluorescent protein (GFP) marker to determine the location of the kinase within maize cells. Then, A fungal assay using Northern Corn Leaf Blight was conducted to determine if the RLK knockouts exhibited differences in average fungal infection time when compared to wild type maize lines B73 and W22. A feeding assay was then conducted using *Spodoptera exigua* to determine if these caterpillars demonstrated increased feeding on the RLK knockout maize lines or the wild type maize lines. All of these experiments generated inconclusive results and require further examination and testing.

Funding acknowledgement: National Science Foundation (NSF)

**P136**

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

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The *defective kernel 5* (*dek5*) locus conditions a severely reduced starchy endosperm with a shrunken phenotype similar to starch biosynthetic mutants such as *brittle1* and *brittle2*. Typical endosperm starch biosynthetic mutants do not affect seedling or plant development. By contrast, *dek5* mutants disrupt embryo development or produce an embryo that develops pale green seedling leaves with occasional white stripes. The pale green seedling phenotype of *dek5* is seedling lethal and suggests the gene is required for chloroplast function. Transmission electron microscopy (TEM) of *dek5* leaf tissue revealed that the mutants have significantly enlarged chloroplasts when compared to normal siblings. These enlarged chloroplasts show a range defective to completely normal internal membrane ultrastructure. Particle size analysis of mid-development starch granules revealed that *dek5* has enlarged starch granules, suggesting *dek5* endosperm amyloplasts are also enlarged relative to normal sibling kernels. *dek5* encodes a 2,123 amino acid unknown function protein with a DUF490 domain. Transient expression of an N-terminal domain of the DEK5 protein fused to GFP suggests the protein is localized to the chloroplast envelope. Consistent with these results, a polyclonal antibody raised against the C-terminal DUF490 domain shows native localization to the chloroplast. Chloroplast subfractionation and protease protection assays further indicate DEK5 is localized to the envelope intermembrane space. Orthologous *dek5* genes are found in the genomes of all completely sequenced photosynthetic organisms. Based on these data, we hypothesize that *dek5* has a role in plastid division and plastid membrane stability. These data suggest plastid division is critical for amyloplast function of the starchy endosperm as well as in the chloroplasts of seedling leaves.

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

**P137**

## The maize RNA Binding Motif48 (rbm48) locus controls endosperm cell differentiation and proliferation

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Correct RNA splicing is essential for eukaryotic gene expression and cell viability. Alternative RNA splicing produces multiple transcripts from a single gene, and the majority of maize genes produce multiple transcript isoforms. We are characterizing RNA splicing factor mutants from the UniformMu transposon-tagging population. Two null alleles in the maize RNA Binding Motif48 locus (*rbm48*) show severe endosperm and embryo developmental defects similar to the maize *rough endosperm3* (*rgh3*) mutant, which encodes a core RNA splicing factor. Histological analysis shows differentiation defects in the basal endosperm transfer layer (BETL), while quantitative RT-PCR indicates that markers for the BETL and embryo surrounding region (ESR) show significant reduction in mRNA levels. Similar to *rgh3*, *rbm48* mutants over-proliferate in endosperm culture even at late endosperm developmental time points indicating that *Rbm48* promotes cell differentiation and represses cell proliferation. The RBM48 protein co-localizes with the core splicing factor U2 snRNP Auxillary Factor (U2AF) and RGH3 proteins in assays using transient expression of fluorescent protein fusions. Bimolecular Fluorescence Complementation (BiFC) analysis suggests RBM48 has physical interactions with the U2AF1 small subunit of U2AF as well as RGH3. RGH3-RBM48 BiFC signal is dependent upon the UHM domain of RGH3 and is consistent with a direct physical interaction based on co-immunoprecipitation results. RNA-seq analysis of mutant *rbm48* and normal endosperm identified a small subset of genes with altered RNA splicing in *rbm48*. These data further support a model for alternative RNA splicing having a central role in endosperm cell differentiation.

Funding acknowledgement: National Science Foundation (NSF)

**P138**

# The Maize Roothairless6 Gene that Encodes a D-type Cellulose Synthase is Involved in Root Hair Elongation

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Root hairs have a significant role in water and nutrient uptake in plants as they provide a substantial increase in root surface area. These epidermal tube-like extensions are thought to play a key role in Zea mays as they are found on all root types of this agronomically significant species. A greater understanding of the pathways contributing to the development of maize root hairs can potentially lead to crop improvement and is the focus of our investigation. Towards this end we genetically mapped, cloned, and characterized the roothairless6 (rth6) gene. This process was facilitated by a combination of two sequence-based cloning strategies, including BSR-seq and Seq-Walking, as well as qRT-PCR, and PCR-based cloning of four independent transposon-tagged mutant alleles. Our findings confirm that rth6 is associated with gene ID GRMZM2G436299, which encodes a D-type cellulose synthase. These findings increase our understanding of root hair development and bring us closer to fully elucidating the pathways at play in root hair development in maize.

**P139**

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

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Gene regulatory networks are central to all cellular processes. In plants, they help link molecular targets with agronomic traits of functional value including biofuel/biomass production, biomaterials, and nutritional health. Transcription Factors (TF) and co-regulators (CoReg) represent ~7% of the maize genome (~3000 genes) and are key regulators of plant metabolic function. To define the gene regulatory networks (GRNs) that regulate metabolism of maize phenolic compounds, we initiated The Grass Transcription Factor ORFeome Project (TFome). We report the development and release of a publicly available maize TF ORF collection (TFome) of 2,034 clones corresponding to 2,017 unique gene models in recombination-ready vectors that enable the facile mobilization of the TF sequences into a number of different expression vectors. The collection also includes several hundred co-regulators (CoREG), which we classified into well-defined families, and for which propose here a standard nomenclature, as we have previously done for TFs. Strategies were developed to overcome the limitations associated with cloning ORFs from an incompletely annotated genome, with a partial full-length cDNA set available, and with many TF/CoREG genes lacking experimental support. This required, in many instances, combining genome-wide expression data with gene synthesis approaches. The strategies developed will be valuable for developing TFomes for other agriculturally important plants. Information on all the clones generated is available through the GRASSIUS knowledgebase (http://grassius.org/), and clones can be requested through the ABRC (http://abrc.osu.edu/). This resource and approach is expected to greatly accelerate the understanding of gene regulatory networks in plants. The maize TFome is now being employed to build a protein-DNA-interaction (PDI) network for the phenylpropanoid pathway. The release of the Maize TFome has been described in Burdo et al., The Plant Journal. 2014 80(2):356-66 and a detailed protocol in Gray et al., Bio-protocol 5(15): e1547. This project was funded by NSF grant IOS-1125620 and by DBI-0701405.

Funding acknowledgement: National Science Foundation (NSF)

**P140**

## The nature of age-related resistance in the maize-CCR1 pathosystem

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One of the most destructive pathogens of maize is *Cochliobolus carbonum* race 1 (CCR1), which can decimate maize plants at any stage of growth. It does so by producing a HDAC-inactivating cyclic tetrapeptide molecule called HC-toxin. Fortunately, the HC-toxin inactivating gene *Hm1* exists in maize that keeps almost the entire maize germplasm immune to this pathogen. *Hm1* confers immunity by encoding an NADPH-dependent HC-toxin reductase (HCTR). While the wild type allele of *Hm1* confers protection in all parts of the plant at every stage of development, forms of this gene exist that confer protection in an age dependent manner, initially conferring little or no protection at the seedling stage but gradually improving with time to confer complete immunity at anthesis. We cloned two of these adult plant resistance (APR) genes: an allele of *Hm1* (*Hm1A*) and a homeolog (*Hm2*). The results revealed that the *Hm1A* HCTR has five amino acid changes, while *Hm2* encodes a truncated enzyme lacking the 52 C-terminal amino acids. Given that their transcriptional and translational levels remain unchanged during development, the APR phenotypes of *Hm1A* and *Hm2* are expected to be dictated post-translationally. We have confirmed weakened HCTR activity in all APR alleles tested by quantifying the amount of HC-toxin reduced by leaf protein extracts. We are also determining the kinetic parameters of the HM1, HM1A, and HM2 enzymes to determine if affinity for the NADPH cofactor is affected in the APR enzymes. To further investigate the potential role of NADPH in defining APR, we have quantified temporal and developmental changes of *in planta* leaf NADPH levels.

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**P141**

# The specific serine phosphorylation of Maize plasmid starch phosphorylase has novel function: regulating proteolysis and phosphorylation-dependent protein-protein interaction

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Starch phosphorylase (SP) reversibly adds glucosyl units from glucose-1-P to the non-reducing ends of α-glucans in plant cells. Genetic evidence reveals that the plastidial form of this activity is involved amylopectin biosynthesis and starch granule formation in several plant species. Its precise role, however, and how SP interacts with other enzymes in the starch biosynthetic pathway, is unknown. Compared to other phosphorylase enzymes, the plastidial form of SP, designated phoL, contains an 80 amino acid insertion (L80) that is involved in starch binding and specific proteolytic cleavage in vivo, but does not appear to be required for normal catalytic activity. L80 contains a PEST sequence predicted to regulate proteolytic degradation, and is known to be phosphorylated on specific Ser residues in sweet potato and maize. This study investigated the role of L80 Ser phosphorylation regarding specific proteolysis of maize phoL and interaction of phoL with other proteins from amyloplasts of developing endosperm. Site-specific changes of maize phoL were generated in which Ser430 and Ser431, within the PEST sequence of L80, were changed to Ala, either in single or double mutants. Expressed mutant or non-mutant phoL were used as bait in pull-down experiments with amyloplast lysates. Several proteins were found to bind to phoL depending on phosphorylation at one or both of those Ser residues. In addition to previously identified binding partners, this study revealed phosphorylation-depending binding to the ADPglucose transporter encoded by the bt1 gene. Non-mutant maize phoL was specifically proteolysed into fragments when exposed to amyloplast lysates. The Ser to Ala single mutants were notably more stable than wild type in such conditions, and the double mutant was fully resistant to proteolysis. These results demonstrate that Ser phosphorylation within maize L80 regulates the interactions of phoL with other starch biosynthetic enzymes, and the proteolytic processing of the enzyme.

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**P142**

## Tolerance To Heat Stress Is Modulated By Changes In The Transcriptome And Lipidome Of Maize

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Maize (*Zea mays*) is the most cultivated cereal crop in the world and it is an important source of energy for biofuels, raw material for industry, and food. To meet the demand for food of the growing world population, it is necessary to increase production in agricultural systems where the yields don't meet their potential due to adverse environmental conditions, such as in tropical and sub-tropical areas. Here, heat stress plays a major role in reducing yields, especially when coupled with drought. In order to better understand the genetic basis conferring heat stress tolerance in maize, further characterization of the genetic and molecular mechanisms is needed. In these studies, we provide the first extensive description of the maize leaf transcriptome and lipidome re-modulation in response to heat stress through RNA-Seq. The transcriptome analysis was conducted in a susceptible (B73) and four heat tolerant (Mo17, B97, CML322, LPS-F32) inbred lines and integrated with comparison of leaf lipid profiles. The common and unique sets of transcripts among the five maize inbred lines are described and related to changes in lipid composition of the cellular membranes after heat stress. We hypothesize that the re-modulation of lipid species is regulated at the post-transcriptional level. Overall our results allow us to formulate a first hypothesis on the gene networks that confer tolerance to heat stress in maize and identify specific candidate genes for future functional characterization.

Funding acknowledgement: USAID

**P143**

## Transgenic expression of DIMBOA biosynthesis genes interferes with plant development and biotic interaction

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Plants produce a vast array of secondary metabolites, many thereof are dedicated to defense. The diversity of defense compounds within the plant kingdom restricts the development of microbial and herbivore generalists. Biosynthesis of secondary metabolites branches-off from primary metabolism. Therefor, any secondary metabolite can be expressed in plants when metabolically connected via the branchpoint enzyme. This allows stacking of different chemical defenses. Benzoxazinoids are defense compounds characteristically found in grasses. The main representative in maize is DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one). As a prerequisite for the transfer of the pathway, all maize benzoxazinoid biosynthetic (Bx-genes) have been isolated. To get first insight into the requirements and interactions of pathway transfer, we introduced the branchpoint enzyme BX1 and the benzoxazinoid specific P450 monooxygenase BX2 into Arabidopsis thaliana. The Bx1Bx2 transgenics synthesize the intermediate indolin-2-one, the concentration is correlated with the expression level of BX1. In Arabidopsis this intermediate, however, is readily modified by hydroxylation and glucosylation. Indolin-2-one and/or its derivative have a dose-dependent impact on plant development. The plants are dwarfs and male and female sterile. Sterility can be avoided if the transgenes are not expressed in the flower. The Bx1Bx2 transgenics are more susceptible to herbivory and powdery mildew. This might be caused by reduced levels of the Arabidopsis endogenic defense metabolite camalexin in the transgenics.

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**P144**

## Transposon mutagenesis and functional analysis of maize genes involved in mycorrhizal signaling pathway

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The majority of the land plants partner with soil dwelling fungi to form arbuscular mycorrhizal symbiosis, through which plants gain physiological and ecological benefit. Successful mycorrhizal association starts with the plant perception of secreted fungal molecules. Plant genes involved in this signaling process have been identified largely through forward genetics in model legume species, but they are still poorly studied in maize. In this study we identified several maize genes that are putatively involved in early and late mycorrhizal symbiosis signaling, including *ZmDMI1* (Pollux), *ZmDMI3*, *ZmNFR5-1*, *ZmNFR5-2*. To characterize the functions of these genes, we conducted maize *Ac/Ds* transposon mutagenesis using a high throughput planting and genotyping platform. In addition, we identified four *ZmNFP1* uniform Mu mutant alleles from maize GDB. F2 progenies of three *ZmDMI1* mutant alleles were inoculated with *Rhizophagus irregularis* and plants were analyzed for the mycorrhizal colonization. Arbuscule formation was completely abolished and fungus infection level was significantly lower in two mutant alleles that harbor *Ds* insertions in the exons. Accordingly, quantitative RT-PCRs revealed that the transcription of *ZmDMI1* is significantly suppressed, likely through non-sense mediated RNA decay. This result suggests *ZmDMI1* pays an important role in the establishment of mycorrhizal association in maize.

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**P145**

## Unequal Recombination at a Disease Resistance Locus in Sorghum

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Crop pathogens are constantly evolving and threatening food production worldwide. Single plants contain up to hundreds of resistance (R) genes, which provide the diversity for new resistance specificities to evolve. However, huge pathogen populations and their short generation times give them a major advantage in mutating to escape any resistance trait. Unequal recombination between R genes and equal recombination between R gene alleles have been shown to be major factors in R gene evolution. Previous studies have shown that unequal recombination between three resistance gene paralogs at the *Pc* locus of sorghum occurs frequently, primarily within an ~560bp hotspot. This recombination hotspot is within the domain responsible for pathogen recognition, which is where recombination events would be most likely to result in new resistance specificities. The purpose of this project is to study meiotic recombination rates and patterns in transgenic maize at the sorghum *Pc* locus as well as at its maize orthologue, the *Rp3* rust-resistance locus.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P146**

## Use of Intrinsic Volatile Compounds for Bird Resistance In Sorghum

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Sorghum is the fifth leading cereal crop grown after rice, wheat, maize and barley. It originated in Northern Africa but is now widely cultivated in the Semi-Arid Tropics and is a main staple in the diet of over 30 countries in Africa and Asia. Like many seed crops, sorghum falls prey to bird predation. In Africa, the Red-billed Quelea alone causes in excess of $50 million in agricultural losses annually; leading to food shortage, malnutrition, and contributes to the poverty of small farmers. Current means aimed at controlling the Red-billed Quelea show little effect as they lack natural predators and are extremely prolific, with ~1.5 breeding pairs which reproduce up to three times annually with an average of 3 eggs a clutch. Methyl anthranilate (MA), a biosynthetic intermediate in the Trp biosynthesis pathway in plants and a widely used food additive, has been shown to be a strong bird repellent and is currently commercially available to farmers (ie.“Bird Shield”). However, MA is photosensitive and must be sprayed repeatedly, thus becoming very expensive and non-feasible for small farmers in Africa. MA is naturally produced in the leaves of sorghum by the conversion of anthranilic acid to MA by a methyl transferase belonging to the SABATH family of enzymes. Our main goal is to produce a safe and effective bird repellent line of sorghum that relies on the production and volatilization of MA in the seed head using timing- and tissue-specific promoters.

**P147**

## Using a kernel culture system to explore the regulatory network for growth responses to nitrogen.

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Increases in agricultural output have been achieved primarily through genetic approaches to crop improvement and application of mineral fertilizers, such as nitrogen (N). N fertilizers, however, require large energy inputs and contribute significantly to pollution. To overcome these issues in the face of increased demands, it is necessary to develop crops with higher N use efficiency (NUE). For genetically complex traits such as NUE, breeders face challenges due competing processes, such as the trade-off between the use of N for vegetative growth and storage for reproductive growth. To gain a better understanding of the genetic factors driving grain yield responses to N in *Zea mays*, my research utilizes a system of culturing kernels *in vitro*, allowing for precise control of N form and availability at different developmental stages. Data accumulated on growth, metabolites and gene expression will permit testing of specific hypotheses of how NUE tradeoffs are governed, i.e. via stoichiometric constraints, co-regulation, or independent interacting network models. Previous experiments on B73 × Mo17 hybrids using this system have already identified several genes that are differentially regulated under various N regimes. Lines containing Robertson’s Mutator transposon insertions in these genes and their promoter regions have been obtained from the UniformMu collection and will be cultured and analyzed. Additionally, the experiment will be expanded to include genotypes known to vary in grain responses to N, such as the Illinois Long Term Selection Experiment for grain protein concentration (i.e. seed nitrogen).

Funding acknowledgement: National Science Foundation (NSF), Illinois Corn Growers Association

**P148**

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

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During daylight, plants often have excess photosynthetic productivity, resulting in the accumulation of photosynthates, including sucrose that is transiently stored in the vacuole. At night, or as photosynthesis becomes limiting because of environmental conditions, plants can remobilize sucrose from the vacuole into the cytoplasm to sustain the plant's metabolism and growth. Based on homology to SUT2 transporter proteins from other plants, maize *Sucrose transporter2* (*ZmSut2*) is hypothesized to be located on the tonoplast membrane and functions as a sucrose/H+ symporter, exporting sucrose that is temporarily stored in the vacuole. To understand the biological roles of *ZmSut2*, we identified several *Mutator* (*Mu*) transposon insertions into the gene. *ZmSut2* mRNA sequence from one of the insertion lines revealed the *Mu* insertion caused a pre-mature stop codon, indicating it is a null mutation. This line was chosen for further biochemical and phenotypic analyses. Under field conditions, we observed *sut2* homozygous mutant plants grew slower, had smaller tassels and ears, and had fewer kernels per ear when compared to wild-type siblings. We also found that the mutant leaves accumulated more sucrose, glucose, and fructose compared to wild type. These findings suggest that *ZmSut2* functions to remobilize sucrose out of the vacuole for subsequent use in growing tissues, and that *ZmSut2* function makes an important contribution to maize development and agronomic yield.

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**P149**

## ZmCCD7/ZpCCD7 encodes a carotenoid cleavage dioxygenase mediating shoot branching

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Carotenoid cleavage dioxygenase 7 (CCD7) functions to suppress shoot branching by controlling strigolactone biosynthesis. However, little is known about CCD7 and its functions in maize and its ancestor (Zea ssp. parviglumis) with numerous shoot branches. We found that ZmCCD7 and ZpCCD7 had the same coding sequence, indicating negative selection of the CCD7 gene over domestication from Zea ssp. parviglumis to Zea mays. CCD7 expression was highly responsive to phosphorus deficiency in both species, especially in the meristematic zone and the pericycle of the elongation zone of maize roots. Notably, the crown root had the strongest ZmCCD7 expression in the meristematic zone under phosphorus limitation. Transient expression of GFP tagged ZmCCD7/ZpCCD7 in maize protoplasts indicated their localization in the plastid. Further, ZmCCD7/ZpCCD7 efficiently catalyzed metabolism of six different linear and cyclic carotenoids in E. coli, and generated β-ionone by cleaving β-carotene at the 9,10 (9',10') position. Together with suppression of shoot branching in the max3 mutant by transformation of ZmCCD7/ZpCCD7, our work suggested that ZmCCD7/ZpCCD7 encodes a carotenoid cleavage dioxygenase mediating strigolactone biosynthesis in maize and its ancestor.

**P150**

## ZmMADS47 regulates zein gene transcription through interaction with Opaque2

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Zeins, the predominent storage proteins in maize endosperm, are encoded by multiple genes and gene families. However, only a few transcriptional factors for zein gene regulation have been functionally characterized. In this study, a MADS-box protein, namely ZmMADS47, was identified as an Opaque2 (O2) interacting protein via yeast-two-hybrid screening. The N-terminal portion of ZmMADS47 contains a nuclear localization signal (NLS), and its C-terminal portion contains a transcriptional activation domain (AD). Interestingly, the transcriptional activation activity is blocked in its full length form, suggesting conformational regulation of the AD. Molecular and RNA-seq analysis of *ZmMADS47* RNAi lines revealed down regulation of α-zein and 50kD γ-zein genes. ZmMADS47 binds the CATGT motif in promoters of these zein genes, but ZmMADS47 alone is not able to transactivate the promoters. However, when both O2 and ZmMADS47 are present, the transactivation of these promoters was greatly enhanced. This enhancement was dependent on the AD function of ZmMADS47 and the interaction between ZmMADS47 and O2, but it was independent to AD function of O2. Therefore, it appears interaction with O2 transcriptionally activates ZmMADS47 on zein gene promoters.

Funding acknowledgement: National Natural Sciences Foundation of China

**P151**

## A genetic screen to identify maize mutants with cell division defects during stomata formation

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Stomata formation in the grasses is an invariant process that requires both symmetric and asymmetric cell divisions. Previous analysis of maize mutants with defects in stomata formation has provided new knowledge about signaling pathways, actin organization, microtubule organization, preprophase band formation, and additional aspects of cell division. We have initiated a screen for new maize mutants with defects in stomatal complex organization due to abnormal cell divisions. We screened 1000 EMS mutagenized F2 families generated by Clinton Whipple and grown by Madelaine Bartlett. We collected leaf tips from up to 20 mature individuals from each family and made glue impressions to view the organization and patterning of the stomatal complexes. We identified 23 families that segregated putative mutants with defects in stomata organization. We are in the process of backcrossing and confirming segregation of the mutant phenotypes in the next generation.

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**P152**

## A maize kernel mutant with impaired phosphatidylinositol 3-kinase-related protein kinase (PIKK) function

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We have characterized a new *defective kernel* (*dek*) mutant in maize called *dek34-Dsg1* with a mutation in a gene that is predicted to encode Tel2-Interacting Protein 2 (TTI2). The mutation is caused by an insertion of an engineered *Ds* transposable element tagged with GFP (Dsg). In humans and yeast, TTI2 has been found to interact with TEL2 and TTI1, forming the TTT complex that regulates the maturation and stability of the phosphatidylinositol 3-kinase-related protein kinase (PIKK) family, members of which are important for organismal development. The *dek34-Dsg1* mutant is lethal, has severely underdeveloped endosperm and embryo, highly reduced seed protein, and lacks some histologically distinct seed compartments. These phenotypes are consistent with mutation in the PIKK member Target of Rapamycin (TOR), which is a major regulator of cell growth and division in response to nutrients. Reciprocal crosses also shows that *dek34-Dsg1* has reduced pollen transmission, a phenotype that is consistent with defects observed in Ataxia Telangiectasia Mutated (ATM) mutants, another PIKK member that is involved in DNA damage repair. Phylogenetic and gene expression analysis of the TTT complex members in maize show that they are single copy genes that have tightly-linked expression profiles, a characteristic of dosage-sensitive genes whose protein products form complexes. Taken together, these results show strong evidence for the role of *dek34-Dsg1* in PIKK function.

**P153**

## Aberrant meiotic modulation partially contributes to the lower germination rate of pollen grains in maize (Zea mays L.) under low nitrogen supply

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Pollen quality is a key factor in determining pollination efficiency and crop yield. Insufficient nitrogen (N) input or low N availability substantially reduces grain yields. How low N affects pollen germination remains a particularly important biological question to be addressed. We found that only low N resulted in a significantly lower germination rate of pollen grains after four-week low N, phosphorus, or potassium treatment in maize production. Importantly, cytological analysis showed 7-fold more mininuclei in male meiocytes under the low N treatment than control, indicating that the lower germination rate of pollen grains was partially due to numerous chromosome loss events resulting from preceding meiosis. Further gene expression analysis revealed dramatic down-regulation of Nuclear Division Cycle 80 (Ndc80) and Regulator of Chromosome Condensation 1 (Rcc1-1) expression, and up-regulation of Cell Division Cycle 20 (Cdc20-1) expression. Aberrant modulation of these key meiotic regulators presumably resulted in high likelihood of erroneous chromosome segregation, as testified by pronounced lagging chromosomes at anaphase I or cell cycle disruption at meiosis II. Together, our data suggest a cytogenetic mechanism of how low N affects male meiosis, and causes a higher chromosome loss frequency and eventually lower germination rate of pollen grains in a staple crop plant.

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**P154**

## Acute, an automated morphological landmark identification pipeline for PlantCV

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Phenotyping plants through developmental time can provide critical insights into genotypic or environmental effects on plant morphology. Interest in developing high throughput phenotyping methods for studying developmental morphology has grown in recent years. While progress in this area has been swift, most protocols focus on extracting gross morphological data that relates to whole plant size and stature rather than the identification of discrete features. To address this lack of capacity to study homologous organs and/or features across development, we have begun to develop a new pipeline within the PlantCV library, to identify informative points (landmarks) across a whole plant image. Presently, our method utilizes a derived form of chain coding in which the contour of a plant is scanned to identify regions of high contrast. Following the identification of these high contrast islands one of several contraction steps can be undertaken to single out the most informative point for each island that can be used to assess homology between developmental stages. This provides a mechanism for rapidly identifying morphological points of interest in time series data to accelerate acquisition steps for homologous point studies. Presently, this method is being developed for the identification of distinct organs within grasses, focusing on the model C4 grass, *Setaria viridis*, although we will be working to develop components of our pipeline in a modular fashion so that they can be applied more broadly to other plant systems.

Funding acknowledgement: National Science Foundation (NSF)

**P155**

## Analysis of maize SWEET transporters

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Maize is intensively cultivated worldwide with more than 1 billion of tons produced in 2013 (FAOstats 2013) and plays a critical role in supporting the growing world population. Therefore, the understanding and improvement of yield potential of crops is essential but also dependent on the efficiency of numerous processes from light interception and photosynthate conversion to storage of these products. Besides their central role in carbon allocation, transporters required for phloem translocation are only partially understood in crops (Bihmidine *et al.,* 2013). In maize, ZmSWEET4c is a hexose efflux transporter involved in sugar transfer from the maternal phloem into the growing kernel (Sosso *et al.,* 2015). *Zmsweet4c* mutant kernels impaired kernel filling results in *"empty pericarp"* phenotype. *ZmSWEET4c* is specific and essential to kernel filling and *zmsweet4c-umu1* mutation induced a dramatically reduced endosperm and the failure to establish the basal endosperm transfer layer (BETL) critical for kernel filling. However, it remains unclear if and how SWEET transporters contribute to sink strength. Furthermore, the effects of the *zmsweet4c-umu1* mutation on the embryo development seem to be minor: homozygous mutant embryos are able to develop into normal seedlings suggesting a different pathway for sugar translocation. In maize genome 23 *SWEET* genes have been found and belong to four distinct phylogenetic clades reflecting the classification established in Arabidopsis (Chen *et al.,* 2010; Eom *et al.* , 2015). To determine the role of SWEET transporters in kernel filling, several *SWEET* genes have been targeted by CRISPR/Cas9 system and transport specificity has been investigated. The tissue-specific localization of these different transporters are studied to determine their role, redundancy and/or compensatory pathways. Together these analyses of ZmSWEET transporters in maize kernel filling may further our understanding of the sugar translocation from source to sink, and provide new insights into the mechanisms and the regulation of carbon allocation.

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**P156**

## Approaches to analyzing rearrangements in the rf3 region of chromosome 2

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CMS prevents normal pollen development due to mitochondrial mutations and is maternally inherited. However, nuclear genes called restorers-of-fertility (Rf) are found in some lines that override this defect. Three types of CMS (T, S, and C) exist in maize. In CMS-S, the pollen grains develop normally until the starch-filling stage, when they degenerate. A high level of a 1.6 kb CMS-S-specific RNA transcript within the mitochondria is associated with this effect. A dominant restorer for CMS-S, Rf3, reverses the male sterility by causing this CMS-associated transcript to be degraded. Previous mapping studies located the rf3 gene to the distal region of the long arm of chromosome 2. The reference genome sequence available for maize is from the B73 inbred line, which does not restore fertility (rf3/rf3). Several lines of maize including Ky21, TR, CI21E and CE1 have dominant Rf3 restorer alleles. These lines were crossed onto the non-restoring (rf3/rf3) Mo17 inbred containing CMS-S mitochondria, and then were continually backcrossed by Mo17 pollen to create near-isogenic lines (NILs) that differ only in the region of rf3. We have been using newly available sequence data from Ky21 to analyze the Rf3-containing region as well as comparing it to the rf3 regions of B73 and Mo17. Comparative bioinformatic analyses suggest that there are rearrangements in this region of chromosome 2.

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**P157**

## Auxin Evo-Devo: Reverse genetic approaches to understanding the role of auxin in shoot development

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Auxin regulates nearly all aspects of plant growth and development. A better understanding of the genes controlling auxin biosynthesis, transport, and perception is therefore fundamentally important to basic plant biology with applications in crop improvement. Previous research has demonstrated both conservation and diversification of the role of auxin in maize and Arabidopsis development. We are using maize vegetative and reproductive development as a model to further understand how auxin regulates development using both forward and reverse genetic approaches.
Phylogenetic analyses of 15 gene families controlling auxin biosynthesis, transport and response illustrates complex relationships amongst monocot and eudicot clades. Reverse genetic analysis has confirmed 85 transposon insertions (from the UniformMu and Mu-Illumina collections) in 51 genes. Higher order mutant analysis is being guided by both phylogenetic and expression analyses. Results from the *vanishing tassel2* (*vt2* ), *ZmPIN*, *ZmTIR/AFB*, *ZmARF* and *ZmAux/IAA* gene families involved in auxin biosynthesis, transport and perception, respectively will be presented.
www.auxinevodevo.org

Funding acknowledgement: National Science Foundation (NSF)

**P158**

## Brace root emergence is regulated by light

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A natural counterbalance to lodging in maize is the formation of stem-born brace roots, yet little is known about how these roots develop. One factor proposed to promote brace root emergence is light [1]. Hébert et al. showed that shaded plants show differential brace root emergence, however it was unclear whether the effects on brace roots were direct or indirect. To investigate the requirement of direct light perception on brace root emergence, we grew the Nested Association Mapping (NAM) [2] founders in the greenhouse with the base of the stem either exposed to light or shaded (95% light filtered). Our results suggest that light signaling directly promotes brace root developmental programs in Maize.

Funding acknowledgement: Gordon and Betty Moore Foundation and the Howard Hughes Medical Institue

**P159**

## Characterization and mapping of the *Suppressor of sessile spikelet 3* (*Sos3*) mutant which functions in paired spikelet development in maize

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*Zea mays* (maize) and rice are two of the most important cereals in the world due to their central roles in agriculture. The spikelet, a short branch which produces florets, is the fundamental unit of grass inflorescences. A difference between these grasses is maize produces paired spikelets while rice produces single spikelets. However, the *Suppressor of sessile spikelet 3* (*Sos3*) mutant of maize produces single instead of paired spikelets, causing defects in the development of the male (tassel) and female (ear) inflorescences. Therefore, the *Sos3* gene may play a role in the evolution of the paired spikelet. *Sos3* mutants are phenotypically characterized by fewer tassel branches and fewer kernels on the ears. Characterization of mutant phenotypes through histology and scanning electron microscopy (SEM) shows that single spikelets are produced in place of paired spikelets, thus indicating that the *Sos3* gene functions in meristem development. To determine the location and identity of the mutated gene, the *Sos3* mutant is being mapped. Linkage analysis with microsatellite markers shows *Sos3* maps to chromosome 1 (bin 6) between markers umc1988 and umc2025, and fine mapping is continuing. Identifying the gene responsible for the *Sos3* mutation will provide valuable insight into spikelet development not only in maize, but also in other cereals, such as rice.

Funding acknowledgement: National Science Foundation (NSF)

**P160**

## Characterization of a DII-based auxin reporter in maize

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Auxins play a pivotal role in multiple aspects of plant development that can influence crop production such as root growth, branching, and inflorescence architecture. For these reasons, learning how auxins modulate maize development may be useful for engineering crops for future agricultural needs. In *Arabidopsis thaliana*, F-box protein transport inhibitor response 1/AUXIN SIGNALING F-BOX proteins (TIR1/AFBs) trigger the degradation of AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) responsive repressors upon auxin perception, allowing transcription of auxin response genes. Domain DII of the AUX/IAA repressor is necessary and sufficient to trigger its auxin-dependent degradation. In this work we used a live cell marker composed of the auxin responsive DII domain from *A. thaliana* IAA28 fused to nuclear localized yellow fluorescent protein variant Venus (DII-VENUS-NLS) and driven by the ubiquitin promoter to track auxin accumulation and response in maize plants. As a proof of principle, we showed that IAA application resulted in a 50% decrease in fluorescent signal in the first 25 minutes and continued degradation over longer times. Moreover, the degradation response was dependent on the concentration of IAA used, and was mediated through the proteasome, since the addition of the proteasome inhibitor MG132 prevented DII-VENUS-NLS degradation even in the presence of IAA. We demonstrated that DII-VENUS-NLS is suitable for analysis of endogenous auxin accumulation, since DII-VENUS-NLS fluorescent signal was reduced in root tips, where auxins accumulate, and in the leaf vasculature through which auxins are transported. Two significant benefits of this marker, compared with other auxin reporters, is that nuclear localization simplifies visualization and quantification in individual cells, and its rapid response to auxin allows analysis of dynamic auxin processes.

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**P161**

## Characterization of the Classic Maize Mutant, *Tasselseed5*

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In maize, selective abortion of carpels in tassels and anthers in ears cause a monecious flowering habit. A classic dominant mutant, *Tasselseed5 (Ts5)*, fails to abort tassel carpels and lower florets of the ear, indicating a failure of sex determination. *Ts5* was first described by Nickerson and Dale in 1955 (Annals of the Missouri Botanical Garden Vol. 42 (1955)) but appears even earlier on a linkage map by Emerson in 1932 (Proceedings VI of the International Congress of Genetics1: 141-152). Mo17 is permissive and B73 is restrictive of the *Ts5* phenotype. Plant height is reduced in *Ts5* mutants and this reduction is due to shortness of internodes above the ear. Mutant stems are wider below the tassel. For floral traits including tassel length, spike length, ratio of feminized branches to the total branch number, and length of the spike that is feminized, homozygotes are slightly more severe. In addition to phenotypic analysis, we present the current status of efforts toward positional cloning of the locus which resides in bin 4.03.

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**P162**

## Characterizing long non-coding RNAs during maize anther development

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To date, functions have been assigned to only a handful of long non-coding RNAs (lncRNAs) in flowering plants. Of these, most were demonstrated to play an important role in development. A required first step in functional characterization is to determine which lncRNAs are expressed in individual organs, tissues, and cell types. Using 10 staged fertile anther transcriptomes from the W23 background, sampling over 30 days of development from the pluripotent anther lobe stage through a few days prior to pollen shed, plus analysis of transcriptomes of sterile mutants defective in one or more cell types at several stages prior to meiosis, we are identifying constitutive, stage-restricted, and likely cell-type specific lncRNAs. We discuss their potential roles in regulating protein-coding genes and specific cell type development. The standard definition of lncRNAs overlooks open reading frames shorter than 100 amino acids, despite the observation that more than 8% of the ~30,000 available full length maize cDNA sequences encode proteins of fewer amino acids, that about 4% encode predicted proteins of fewer than 50 amino acids, and that there are dozens of documented short proteins with specific roles in plants. To determine whether categorized lncRNAs are actually protein-coding mRNAs, we have interrogated the lncRNAs for shorter reading frames and determined whether the predicted proteins are present in the maize anther small protein proteome just prior to and during meiosis. In this poster we will present preliminary findings from this study.

Funding acknowledgement: National Science Foundation (NSF), the International Postdoctoral Exchange Fellowship Program

**P163**

## Comparative transcriptomics of large, medium and small maize shoot apical meristems

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The maize shoot apical meristem (SAM) is a pool of stem cells that give rise to above ground tissues. We have observed heritable morphological differences in SAM size and shape among maize inbred lines. These SAM morphological variations correlate with agronomical important traits such as flowering time, internode size, plant height and leaf number. Further exploration of the molecular genetic mechanisms underlying the regulation of SAM morphology may also contribute to a better understanding of plant architecture. Previous GWAS of maize SAM morphometric variation revealed associated genes functioning in auxin transport and cell size/division, yet no master regulatory genes were identified. To date, no comparative transcriptomic study has been done to explore the quantitative transcriptional differences correlating with difference in SAM size and shape. In this study, we selected nine maize inbred lines from our previous study among three categories, corresponding to small, medium and large SAMs. We used laser-microdissection (LM) on two-week old maize seedlings to precisely sample SAM-specific RNA, excluding the transcriptomes of other tissues. After linear amplification of extracted SAM RNA, Illumina based RNA-seq identified mRNAs that are implicated in the regulation of SAM morphological variation in maize. This study provides a transcriptomic dataset that is amenable to genetic analyses of SAM morphometric variation.

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**P164**

## *Control leaf angle 1 (Cla1)*, a major QTL controlling leaf angle in maize

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Upright maize leaf architecture has been selected by plant breeders for higher planting densities and maize varieties with an erect leaf angle (LA) show increased grain yields. Recent GWAS surveys have identified several quantitative trait loci (QTL) for LA, including two that mapped to known regulators of ligule development (Tian et al. 2011 Nat Genet 43: 159-162). In our recent study *ZmCla1*, that accounted for 35.7 % of leaf angle variance in our mapping populations, was identified and isolated by fine mapping and positional cloning. Sequence analysis revealed promoter variation in *ZmCla1* between the two QTL mapping parents. Association studies further suggested that promoter variation in *ZmCla1* is responsible for the phenotypic variations in leaf angle across a diverse maize population. Transgenic maize plants carrying a *ZmCla1* RNAi cassette displayed a decreased LA compared to near isogenic wild-type plants and over-expression lines showed an increased leaf angle, indicating that ZmCla1 promotes a more outright leaf angle. Here we present both genetic and molecular data to define the function of *ZmCla1* and suggest new opportunities to improve maize yield through leaf angle associated molecular breeding.

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

**P165**

## Cytokinin decreases leaf growth by reducing cell division

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Maize leaves grow through coordination of the basic cellular processes of division and expansion which are organized linearly into distinct growth zones. The growth zones (GZs) are organized into five distinct regions from base to tip, consisting of the division zone (DZ), transition zone 1 (TZ1), elongation zone (EZ), transition zone 2 (TZ2) and maturation zone (MZ). This distinct spatial organization makes the maize leaf an ideal model system in which to study growth regulation at the molecular and cellular levels. Our analysis of the semi-dominant mutant *Hairy Sheath Frayed1* (*Hsf1*) indicated signaling of the plant hormone cytokinin (CK) can alter leaf size. *Hsf1* mutants hypersignal the presence of CK in developing leaf primordia resulting in plants with shorter and narrower leaves at all stages of growth compared to wild type sib plants. The reduction in *Hsf1* leaf size can be reproduced in non-mutant inbred seedlings by a transient treatment of germinating seeds with exogenous CK. The degree of leaf size reduction was dependent on both CK concentration and length of treatment. To further investigate the mechanism of CK-growth reduction, the spatial organization of the growth zones of leaf #4 from *Hsf1* and sib wild type seedlings were analyzed. The smaller leaf size if *Hsf1* plants resulted from a reduced growth rate which was attributed to a reduction in the number of dividing cells and smaller DZ. The fact CK could act as a growth restrictive signal on cell division was surprising, since CK generally promotes cell proliferation. Additional molecular and physiological studies will be presented that suggest CK may negatively influence another growth-promoting hormone at the leaf base to limit the size of the DZ.

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**P166**

## Deciphering how the Glutaredoxin Gene, MSCA1 regulates shoot meristem development through modifying its putative target proteins

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Plant architecture is critical for plant growth and reproductive success. The pluripotent stem cells, resident at the summit of the shoot apical meristem (SAM), are responsible for the vegetative and reproductive architecture. Meristem development is precisely controlled by multiple mechanisms and signaling networks, including the CLAVATA (CLV)-WUSCHEL (WUS) negative feedback loop, auxin and cytokinin signalling pathways, and KNOX pathways. Nevertheless, additional regulators controlling shoot meristem growth are yet to be discovered. Maize (Zea mays) *Aberrant phyllotaxy 2 (Abphyl2)* is a gain-of-function mutant showing enlarged shoot meristems and a decussate phyllotactic pattern. Previous studies in our lab show that *Abphyl2* mutation is caused by transposition of a glutaredoxin gene, *MALE STERILE CONVERTED ANTHER1 (MSCA1*), resulting in altered expression pattern of *MSCA1* in *Abphyl2*. Consistently, *msca1* loss-of-function mutants have reduced meristem size. The growth defect of meristem in the mutants *Abphyl2* and *msca1* suggests a potential novel function of glutaredoxins in meristem development. Interestingly, MSCA1 interacts with a TGA transcription factor, FASCIATED EAR4, in yeast-two hybrid and BiFC assays. Agreeing with the protein interaction, the co-localization of FEA4-RFP and MSCA1-YFP in the nucleus was observed in some cells of SAM and inflorescence meristem. *fea4* loss-of-function mutants exhibit fasciated ears and tassels with greatly enlarged vegetative and inflorescence meristems. Genetic analysis showed that *fea4* is epistatic to *msca1* with respect to SAM size supporting that they interact in a pathway. Here, we propose a novel regulatory module for regulating shoot meristem size, in which the activity of FEA4 is controlled by MSCA1. By using biochemical approaches, we are investigating how MSCA1 regulates the function of FEA4 and other putative targets through post-transcriptional modification. We are also using CRISPR technology to knockout two *MSCA1* close homologs to further investigate the function of glutaredoxin genes in meristem development

Funding acknowledgement: National Science Foundation (NSF), NIFA

**P167**

## Developing a mathematical modeling approach to maize cell division

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Cell division is critical for the growth of any organism as is regulation of when and where cells divide. One aspect of cell division that plays a critical role in proliferation and development in plants is the spatial control over cytokinesis. Although there has been recent progress in understanding regulation of asymmetric division plane orientation, less is known about general mechanisms of proliferative or symmetric division plane orientation. We developed a mathematical modeling approach to predict the division of any symmetrically dividing cell. Using maize epidermal cells expressing a live cell marker for microtubules and stained with propidium iodide to outline the cell wall, we extracted the 3D surfaces of both the cell and the preprophase band (PPB), a structure that faithfully predicts the future division site of plant cells. This cell surface was then imported into Surface Evolver which cut the cell volume into two equal volumes while also minimizing the area of the newly formed wall. Surface Evolver produced division surfaces that match observed symmetric divisions. In other words, when these predicted divisions were compared with the location of the PPB, many were closely aligned. Intriguingly, when significant discrepancies were observed between the PPB location and the predicted division, an adjacent cell had a cell wall or a PPB parallel to the predicted division. These were clear examples of an observed but poorly understood phenomenon known as avoidance of a four-way junction. More than half of the dividing maize epidermal cells we observed avoided creating four-way junctions indicating its important role in division plane orientation. Future research will focus on improving predictions based on information from nearby cells as well as developing a more probabilistic approach to division plane predictions.

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**P168**

## Differential gene expression in the upper and lower floral meristem of maize

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Maize spikelets contain two florets, which are the product of the upper and lower floral meristems. The two florets are morphologically indistinguishable, however mutant analysis and limited gene expression studies indicate that the gene regulatory networks in the upper and lower floret are distinct. For example, mutations in the MADS-box transcription factor, *bearded-ear (bde)*, result in meristem indeterminacy in the upper floret and loss of floral meristem identity in the lower floret, suggesting that BDE regulates different genes in the two florets. To examine gene expression in the upper and lower floral meristems, we used laser capture microdissection (LCM) to specifically isolate upper and lower floral meristems and performed RNA-seq from three biological replicates. We identified ~160 differentially expressed genes between the upper and lower floral meristems (≥ 2 log2-fold-change, $q<0.05$); 88 genes showed higher expression in the upper floral meristem and 73 showed higher expression in the lower floral meristem. Importantly, two MADS-box genes that are detectable only in the upper floret by RNA in situ hybridization were enriched more than 10-fold in the upper floral meristem in our data. We are currently examining expression of additional differentially expressed genes by RNA in situ hybridization.

Funding acknowledgement: North Carolina Biotechnology Center

**P169**

## Dissecting the function of potential kranz anatomy regulators in C4 grasses using CRISPR/Cas9 in Setaria viridis and Ac/Ds and Mu mutagenesis in maize

(submitted by Carla Coelho <ccoelho@danforthcenter.org>)
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C4 grasses, such as maize and sugarcane, are the most photosynthetically efficient crops in the world. This productivity is a consequence of both biochemical and anatomical adaptations, and although the biochemistry is well established, the regulatory networks underlying kranz anatomy are largely unknown. It has been suggested that a regulatory network involving the transcriptional regulation of SCARECROW/SHORT-ROOT (SCR/SHR) and the INDETERMINATE DOMAIN (IDD) transcription factors acts to determine cell identity in the leaves of C4 species (Slewinski, 2013). Four members of the IDDs are co-expressed in the leaf gradient and are enriched to the mesophyll (M) cells, and two candidates are bundle sheath (BS) enriched. To functionally dissect the roles of these IDD gene family members, Y1H and Y2H assays were performed to define an interaction network of SHR/SCR and IDD members. Mutants are being generated using available Ac/Ds and Mutator populations in maize and CRISPR/Cas9 in Setaria viridis, a model plant for C4 grasses. Two alleles were identified in two mesophyll-enriched IDDs using Ac/Ds remobilization and one allele in a bundle sheath-specific IDD gene was identified in a Mutator line. As a proof of concept, the Setaria ID1 ortholog was disrupted using CRISPR/Cas9 in Setaria viridis and we obtained 6 independent biallelic events in the first generation. All the biallelic events were late flowering, consistent with the phenotype expected in maize. Three other IDD genes were cloned into CRISPR constructs to generate loss of function alleles. We propose a model in which various IDD family members can act as activators or repressors of BS/M differentiation, depending on interactions with complex members; and we also present, for the first time, that the CRISPR/Cas9 system allows for rapid and efficient gene editing in Setaria viridis.

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**P170**

## Elucidating the genetic networks controlling formation of lateral organ initial cells

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Plant shoot meristems function in both lateral organ initiation and stem cell maintenance. Organogenesis occurs from lateral initial cells in the meristem periphery, whereas stem cells are replenished in the meristematic central zone. The mechanisms whereby lateral organ initial cells are organized from the peripheral zone of shoot meristems are poorly understood in grasses. This study will compare and contrast the mechanisms of lateral organ initial cell organization versus meristematic stem cell organization. Specifically, we aim to answer two central questions: (1) Does the organization of stem cells and lateral organ initials involve the same general genetic network in distinct meristematic domains, or in contrast, are these meristematic functional zones organized by distinct, domain-specific mechanisms; and (2) What gene functions operating within the lateral organ initial-cell organizing center can be exploited to impact organ size? Here, we propose the elucidation of interactive co-expression networks for a deep understanding of the molecular mechanisms underlying the establishment of lateral organ initial cells in plant meristems. These regulatory networks have the potential to identify targets for precision plant breeding of more robust grass leaves and floral organs that may contribute to increased crop yield.

**P171**

## EMB15 functions in plastid 30S ribosome assembly and embryogenesis in maize

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The embryo defective (emb) mutants account for a major group of seed mutants in maize which display specifically arrested embryogenesis with normal endosperm development. Owing to difficulty in cloning, many of the *Emb* genes have not been identified, letting alone elucidation of their molecular functions. Here we report the cloning and functional characterization of *Emb15*. The *emb15* mutant in W22 background displays *emb* phenotypes. *Emb15* was cloned by transposon-tagging and confirmed by multiple alleles. EMB15 contains two domains, an N-terminus domain with high similarity to prokaryotic RimM protein and a C-terminus domain with high similarity to UDP-GlcNAc pyrophosphorylases (UDPGP) in higher plants. *Emb15* appears to derive from fusion of two genes as moss and lower species host the two domains in two separate proteins. The RimM protein in *Escherichia coli* is implicated in assembly of 30S ribosome and is essential for growth. UDPGP is considered to catalyze a reversible reaction of UTP and GlcNAc to PPi and UDPGlcNAc, the precursor of N- and O-linked glycosylation. EMB15 was localized in the chloroplast and nucleus. Expression of *Emb15* in ΔrimM mutant in *E. coli* partially restores the growth rate, suggesting that EMB15 has a similar function of RimM in facilitating 30S ribosome assembly. The function of UDPGP domain is under study.

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**P172**

## Factors affecting on the ratio of types of morphogenesis in vitro in different maize heterotic group

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The viability of the regenerats after repotting from in vitro conditions to soil depends on the types of morphogenesis by which formation of regenerants occurs in callus culture in vitro. Though morphogenesis with future regeneration can occurs through organogenesis or embryoidogenesis (somatic embryogenesis), but the survival rate in vivo of regenerants that were obtained through embryogenesis considerably higher than through organogenesis.
The genotypic features of morphogenesis and regeneration in vitro of five maize inbreds (ДК267, ДК6080, ДК420-1, ДК298 and ДК3070) of breeding perspective for Ukraine Lancaster heterotic group compared to inbreds of PLS61, A188 and Chi31 as representatives of their respective heterotic groups were studied. We investigated also the influence of sucrose concentration in the medium for callusogenesis and the influence of physiological active substance such as 6-benzylaminopurine, indolebutyric acid and cefotaxime in the medium for regeneration on the morphogenesis of these inbreds.
Lancaster group inbreds are able to regeneration the plants either by organogenesis or by somatic embryogenesis, while the regeneration by organogenesis prevails at the others geterotic groups. 30 g/l sucrose provoked the embryoidogenesis at the level 18,8% among Lancaster heterotic group and 11,0% among others heterotic groups and 60 g/l sucrose enhanced the level of somatic embryogenesis to 54,2% and 15,0%, accordingly. 6-benzylaminopurine (0.1 g/l) induced the embryoidogenesis at the level of 43.3% among inbreds of Lancaster heterotic group and 15.6% among others heterotic groups. When using indolebutyric acid (1.0 g/l) the level of embryoidogenesis was reached 40.0% among inbreds of Lancaster heterotic group and 14.6% among others heterotic groups, and cefotaxime (150 mg/l) provided the embryoidogenesis at the level of 23.1% and 15.2% respectively.
Thus, the ratio of types of morphogenesis such as organogenesis and embryoidogenesis is determined by explant genotype, sucrose concentration in medium for callusogenesis and phytohormone composition of medium for regeneration.

**P173**

## Functional analysis of microtubule-associated protein ZmGLR

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ZmGLR encodes a protein with 210 amino acids. The protein sequence is rich in glutamic acid and lysine, and contains VVEKK/EE imperfect repeats, which assembles the putative microtubule binding domain of MAP18.

The N-terminal sequence of 25 residues of ZmGLR has the ability to localize the fusion protein with green fluorescent protein to the plasma membrane, and the deletion of the 2-25 amino acid resulted in the cytoplasmic localization of ΔD2-25ZmGLR. Binding capacity of ZmGLR to phosphatidylinositol phosphates (PtdInsPs) was tested with PIP StripsTM, we found that ZmGLR interacts with $PtdInsP_1$, $PtdInsP_2$ and $PtdInsP_3$. Mutational analysis revealed that N-terminal truncated ΔD2-25ZmGLR could not interact with these PtdInsPs. Co-sedimentation assay, fluorescent co-location and negative staining assay indicated that the ZmGLR can bind to MTs in vitro.

The ZmGLR over-expression and RNAi vectors were constructed and introduced into maize calli using particle bombardment. Abnormal epidermal cells were observed in both ZmGLR overexpression and RNAi plants. In wild-type, pavement cells form a regular undulating pattern of lobes and the projections from neighboring cells interdigitate to form a zipper-like interface. However, in ZmGLR RNAi plants, leaf epidermal cells shorten and fail to promote lobe outgrowth. This result indicated that, as a microtubule binding protein, ZmGLR is involving in organization and coordination of the cytoskeletal components and affecting on the differential growth of a cell.

Funding acknowledgement: National Natural Science Foundation of China

**P174**

## Genome-Wide Analysis of Small RNA-Controlled Gene Networks in Shoot Apical Meristem Function

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In plants, stem cell niches serve as a stable source of cells for postembryonic growth and development. The shoot apical meristem (SAM) gives rise to all aerial organs of a plant, and its activity throughout the plant's lifetime therefore has to be tightly controlled in a spatiotemporal manner. To gain insight into gene regulatory networks behind stem cell maintenance and organogenesis, we generated a high-resolution gene expression atlas of 10 distinct domains within the vegetative maize shoot apex using laser microdissection and RNA deep sequencing. We identified cell type specific expression features and determined unique transcription factor signatures that, next to the interplay of hormones, distinguish stem cells from their differentiating descendants within the SAM. In addition we generated small RNA and degradome deep sequencing data that inform on the role of miRNAs in the maize shoot apex. This showed a subfunctionalization of miRNA family members across the SAM subdomains, and predicts the presence of mechanisms that further fine-tune the accumulation and activity of select small RNAs to regulate key meristem genes.

**P175**

## Grasses suppress shoot-borne roots to avoid water loss during drought

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Many of the major crop plants are in the Poaceae family, which develop fibrous root systems characterized by a high-degree of root initiation from the basal nodes of the shoot. While this post-embryonic shoot-borne root system represents the major conduit for water and nutrient uptake and mechanical support in grasses, little is known regarding what effect water availability has on the development of this part of the root system. Here we demonstrate that in the model C4 grass Setaria viridis, these basal nodes, often referred as the crown region, locally senses water availability and suppresses post-emergence crown root growth under water deficit. This response occurs in field and controlled environments and was observed in all grass species tested. Luminescence-based imaging of soil-grown root systems revealed that primary-root derived branches proliferate when crown root growth is suppressed. Zea mays and Setaria italica, which are the domesticated relatives of the wild sub-species teosinte and S. viridis, respectively, show less of a reduction in crown root number under water deficit suggesting that this response may have been a target of human selection. Enhanced water status of maize mutants lacking crown roots suggest that this trait may be a useful target for breeding efforts to improve productivity with reduced water inputs.

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

**P176**

## *Hoja loca* is an AUX/IAA mutant that affects lateral organ initiation

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*Hoja loca* mutants have defects in lateral organ initiation and leaf morphology. The mutation produces plants with a wide range of severity, from nearly shoot-less individuals to others displaying no visible phenotype. Mild mutants have leaf defects including the absence of a midrib and fused leaf margins that result in a tube leaf. More severe mutants will also fail to initiate leaves at one or more nodes. Both of these phenotypes suggest aberrant formation of lateral organs from the meristem, disrupting the formation of medial/lateral asymmetry or altogether failing to initiate an organ.

*Hoja loca* is caused by an EMS-induced lesion in ZmIAA38 resulting in a G to E mutation in the core degron region. Identical mutations in other species' IAAs result in a stabilized protein that cannot be degraded in response to auxin signaling. Differential gene expression shows suggests that *Hoja loca* is altering flavonol dynamics. Flavonols affect auxin patterning in the root and lateral root initiation, suggesting that *Hoja loca* may be disrupting a similar process in the shoot.

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

**P177**

## Identification and mapping of a novel *tasselseed* mutation in maize.

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Unisexual maize flower originates through selective abortion of female primordia in the tassel and of male stamens in the ear from an original bisexual inflorescence. tasselseed mutations are known to alter the usual sex fate allowing carpel survival in the male inflorescence and the development of perfect flowers. Objective of the present research is to describe and map a novel tasselseed phenotype carried by an inbred line (Rig7) identified among a set of lines derived from in-vitro culture. In the cross B73 x Rig7, the tasselseed phenotype appeared under the control of two loci. We applied SNP (50K-Illumina)-based bulk segregant analysis, which confirmed the involvement of two loci on chr. 2 and 6. A strong and unexplained reduction in recombination across chr. 2 precluded the characterization of the locus on such chromosome. On the contrary, the locus on chr. 6 was localized in < 2 Mb region on bin 6.07. Further fine mapping analyses on 2,000 F2 recombinants enabled us to narrow the region to approx. 400 kb, which included twelve coding sequences. Candidate genes are being further characterized by searching and testing for additional recombination events and genomic mutations, and by analysis of differential gene expression.

**P178**

## Identification of a Unique Signature for Black Layer

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Physiological maturity is reached at a developmental stage called back layer, where photosyntates are no longer able to move from photosynthetic tissues into the developed grain. Currently there is no high-throughput method available for determining physiological maturity in maize across a large number of experimental plots or across a heterogeneous landscape. Remote sensing with unmanned aerial vehicles (UAVs) may offer a solution to this problem. In this study a fixed wing UAV with multispectral RG-NIR and BG-NIR sensors was used to capture physiological changes caused by prematurely terminating the primary sink. As black layer is the natural termination of the primary sink, prematurely terminating the sink may mimic the physiological consequences of that process. Sink termination was induced in 4-row plots over a 5-wk period by manually removing all developing ears within a plot. The UAV was flown weekly with different sensors, and these images were used to investigate reflectance differences. By applying the normalized difference vegetative index (NDVI), differences in photosynthetic activity for the treatments was observed. This work was done to determine if there is a reflectance signature associated with the loss of a carbon sink during maturation, which could act as a signal for black layer formation.

Funding acknowledgement: Canadian Foundation for Innovation (CFI), Natural Sciences and Engineering Research Council (NSERC), The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)

**P179**

## Identification of KASH and other SUN-interacting proteins in maize

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Meiosis a highly dynamic process where chromosomes undergo active movements to find their homologs for recombination during prophase I. In most species, the movement of meiotic chromosomes is associated with telomere clustering on the nuclear envelope, producing a unique nuclear structure called the bouquet. Inner nuclear membrane resident SUN (Sad1/UNC -84) domain proteins tether meiotic prophase telomeres to the nuclear envelope through specific protein complexes. SUN proteins, highly conserved across kingdoms, interact with outer nuclear membrane resident KASH (Klarsicht/ANC-1/Syne homology) domain proteins to form a physical linker that connects nucleoskeleton to the cytoskeleton. This Linker of Nucleoskeleton to Cytoskeleton (LINC) complex transduces mechanical forces leading to chromosome movements. The identity and function of most LINC complex proteins remain largely unknown in plants. We produced peptide antibodies to ZmSUN2, a C-terminal SUN domain protein of maize, and found that it stains a novel meiotic prophase nuclear structure called the meiotic *SUN belt* (Murphy *et al* 2014, doi:10.3389/fpls.2014.00314). The meiotic SUN belt changes during prophase progression, overlaps with the telomere bouquet, and can be genetically altered by meiosis specific mutants. To learn more about the functions and mechanisms of SUN protein complexes in plants, we are taking a biochemical co-immunoprecipitation approach to pull down SUN and SUN-interacting proteins. We are also cloning candidate KASH genes to be tested for direct targeted interaction assays with SUN proteins. These studies are expected to further define SUN-KASH protein complexes in maize and shed light on the role of the NE in coordinating cytoplasmic forces with chromosomal dynamics.

Funding acknowledgement: National Science Foundation (NSF), American Heart Association (AHA)

**P180**

## Identification of novel molecular components involved in the tillering regulation network of Maize

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Shoot branching or tillering is an important agricultural trait that affects grain yield and biomass production. Commercial maize inbreds produce no tiller or few tillers. However, teosinte, the wild ancestor of maize, shows profound tillering phenotype. *teosinte branched1* (*tb1*) and *grassy tillers1* (*gt1*) are two of the major genes that have been selected during domestication of maize from teosinte to increase the apical dominance of maize main stalk and modulate maize tiller numbers. Mutants with loss-of-function of *tb1* or *gt1* exhibit increased tiller numbers. Both *tb1* and *gt1* act in a pathway to regulate tiller development in response to shade signals. To explore the additional genes involving in the *tb1-gt1* tillering network, we performed EMS-mutagenesis to screen potential suppressors and enhancers of *tb1* and *gt1*. In total, we identified 35 *tb1* and 4 *gt1* of potential suppressors, and 3 *tb1* and 1 *gt1* potential enhancers. Currently we are working on the cloning and characterization and of these suppressors and enhancers.

Funding acknowledgement: National Science Foundation (NSF)

**P181**

## Immunostaining of ZmNDPK1, a G4-DNA-binding protein, reveals various nuclear and cytoplasmic localization patterns in maize tissues before and after experimental flooding to induce hypoxia.

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G-quadruplex (G4) DNA elements are non-duplex DNA structures that occur within chromosomes and at telomeres. G4 DNA is linked to genes associated with cancer and cell-growth in humans but is not well characterized in plants. A recently survey of the maize (*Zea mays* L.) genome for non-telomeric G4 motifs detected ~ 150,000 of them (Andorf *et al.*, 2015 *J Genetics Genomics* 41:627-647). G4 motifs were enriched in genes associated with energy stress signaling and pathways. A nucleoside diphosphate kinase (ZmNDPK1) was recently described as the first high-affinity plant G4-binding protein (Kopylov, Bass, & Stroupe, 2015 *Biochem* 10;54(9):1743-1757). In order to see if ZmNDPK1 was located in nuclei or responsive to hypoxia, we made antisera for cell staining using epifluorescence 3D microscopy. For hypoxia treatments, seedlings were flooded overnight, followed by harvest, fixation, and sectioning for cell staining. A variety of NDPK-staining patterns in slides prepared from normal and flooded plants were observed, including diffuse cytoplasmic signals, small speckles, larger foci, and even long ribbon/tube-like structures. Nuclear staining was generally weaker, but could be seen in some cell types, consistent with a possible nuclear G4-related function. Interestingly, non-hypoxic tissue were slightly more likely to show nuclear staining, though faint and not yet fully quantified. Nuclei from endosperm, a naturally hypoxic tissue, showed nuclear speckle-like staining in some cells. The variety of staining patterns and implied dynamics are consistent with what is known in other species for this highly conserved enzyme. The role of NDPK1 in stress response and G4-DNA-related processes remains an important unanswered question. Given that a human NDPK homolog, NM23-H2, is implicated in cancer biology and G4-DNA binding, these studies have the potential to shed light on fundamental biological functions with broad implications for both agriculture and human biology.

Funding acknowledgement: National Science Foundation (NSF)

**P182**

## Improving orphan African grain crops using the *teosinte branch1/grassy tillers1* and *branched silkless1/indeterminate spikelet1* pathways

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Several orphan African grain crops such as Teff and millet are heat, drought and flooding tolerant, display better water use efficiency, require minimal nitrogen inputs and are nutritionally superior to maize. These plants can be viable alternative crops, especially on marginal croplands not suitable for maize growth. Before such crop plants can be fully utilized in our country, however, further modifications are required in their plant architectures to make them more attractive to North American farmers. For example, Teff plants overproduce tillers with weak stems, resulting in significant grain loss due to lodging. In addition, Teff spikelets are highly indeterminate, producing multiple florets of which only a few can produce seed. By reducing tiller number and floret number, extra resources will be available to the plant to produce more robust seed. Such plants will also be more amenable to row planting and mechanical harvesting.
Previous studies have shown that a pair of plant domestication pathways that were first identified in maize controls the tillering and floret number traits in multiple grasses. For example, the transcription factor teosinte branch1 (tb1) directly binds to the grassy tillers1 (gt1) promoter to activate its expression and repress tillering. The maize AP2 transcription factor indeterminate spikelet1 (ids1) is important for limiting the number of florets made per spikelet, while the branched silkless1 (bd1) ERF transcription factor functions as the switch that allows the spikelet to produce florets. Thus, by manipulating these two pathways we can potentially reduce tiller and floret number and achieve the ideal plant architecture required by modern farmers.
To repress tillering, we are attempting two approaches using transformation protocols we have developed for Teff. First, we are overexpressing the tillering repressors tb1 and gt1, but also putative activators of tb1. Finally, to reduce spikelet indeterminacy and floret number, we are overexpressing microRNA resistant versions of the maize indeterminate spikelet1 gene. These plants will be used as tools to point breeders towards specific genes worthy of selection and hopefully help establish Teff as a viable alternative crop for the developing world.

Funding acknowledgement: National Science Foundation (NSF)

**P183**

## Induced and natural variation in genes encoding the microtubule severing ATPase, katanin p60 (*KTN1*), alter meristem shape, plant morphology and spikelet density

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Cortical microtubules (CMTs) facilitate anisotropic growth by templating parallel rings of cellulose microfibrils, via a *KTN1*-dependent mechanism, around elongating cells that restrict the direction in which a cell can expand. We mapped the semi-dominant maize mutant *Clumped tassel1* (*Clt1*) to a *KTN1* homolog on Chr 8 (*Zmktn1a*). Strongly resembling loss-of-function katanin mutants in Arabidopsis, *Clt1* plants show reduced height, smaller organs and increased spikelet density that is accompanied by a wider tassel meristem. Transforming the maize *ktn1a-Clt1* allele into wildtype Arabidopsis causes shorter and wider siliques, and it is unable to complement a *ktn1* mutant. Conversely, the *ktn1a-B73* allele has no effect in wildtype Arabidopsis and can complement *ktn1* mutants. An additional maize nonsense allele, *ktn1a-dcd3*, produces phenotypes reminiscent of *Clt1* homozygotes when combined with *ktn1a-Clt1*.
An enhancer/suppressor screen crossing *Clt1* with the NAM inbred parents revealed *Clt1*/+ plants with a novel phenotype, showing even shorter plants and compressed upper internodes. Unlike the wider tassel meristem and increased spikelet density in *Clt1*, these compressed internodes develop from SAMs that are narrower than normal. In each case, the enhancer mapped to a Chr 3 interval containing *ktn1b*, the homoeolog of *ktn1a*. Consistent with *ktn1b* underlying this modifier, inbreds causing the enhanced phenotype each had independent *ktn1b* alleles with deleterious splicing defects, while inbreds not showing compressed upper internodes produce intact transcripts. Because the phenotypes of the *ktn1a-Clt1* mutant, the *ktn1a-Clt1*/+; *ktn1b-NAM* double mutants and the NAM inbred parents carrying the enhancer alleles are all distinct from each other, we hypothesize that *ktn1a* and *ktn1b* functions have diverged, but remain partially redundant.

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

**P184**

## Is the ligule involved in Sugarcane mosaic virus movement?

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Virus move a long distance to establish a systemic infection surpassing architectural boundaries in the plants. Maize isolates from sugarcane mosaic virus can systemically infect maize across the ligule (a proposed boundary), at the blade to sheath junction. Initial results point out to a differential accumulation of viral intermediates of replication in this structure as well as of viral particles in virus resistant and susceptible maize lines. Therefore, a discrimination or regulation of viral components might be taking place here. In order to understand if the ligule is an obstacle or a facilitator for virus movement, we are using liguless maize lgl1 and lgl2 mutants which completely or partially lack this structure. So far, we have found a differential response toward viral infection in these two types of mutants and will show the first results of viral localization of the structural viral coat protein in this tissue, along with the phenotypes resulting from the viral infection.

Funding acknowledgement: United States Department of Agriculture (USDA), CONACYT, Mexico

**P185**

## Loss of function of the (*E*)-ß-caryophyllene synthase in most American maize lines is due to a nonfunctional promoter

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The production of volatiles, especially terpenes, is essential for the indirect defense of plants. Maize roots attacked belowground by the larvae of the coleopteran *Diabrotica virgifera virgifera*, emit the insect-induced plant signal, (*E*)-ß-caryophyllene, which strongly attracts an entomopathogenic nematode. Most European maize lines and their wild ancestor, teosinte, release (*E*)-ß-caryophyllene in response to this maize pest. However, most North American maize lines are not able to respond to the larvae attack and may have lost this important defense signal during the breeding process. Recently, we identified the terpene synthase 23 (TPS23), which is responsible for the production of the defense compound (*E*)-ß-caryophyllene.
In our effort to study the molecular regulation of *tps23* between European and American maize lines, we used Nested Association Mapping (NAM) and Genome Wide Association Study (GWAS). The variation of volatile production of the 5000 inbred lines within the NAM population enabled us to identify a significant QTL which is responsible for the production of the sesquiterpene (*E*)-ß-caryophyllene. Further calculation using GWAS resulted in a SNP within the proximity of *tps23*. Comparison of the open reading frames of *tps23* from (*E*)-ß-caryophyllene-producing maize lines and non-producing lines indicated that the QTL does not correspond to the structural gene itself.
Herbivore induced expression analysis of the possible regulatory factors gave no significant conclusion. We therefore focused our investigations on the promoter of the (*E*)-ß-caryophyllene synthase using a heterologous expression system in Arabidopsis thaliana. The results indicate that the loss of (*E*)-ß-caryophyllene production after herbivory in most American maize lines is due to sequence alterations within the promoter of the (*E*)-ß-caryophyllene synthase.

Funding acknowledgement: CRC648

**P186**

# Maize Cell Genomics: a two component transactivation system as a tool for maize functional genomic study

(submitted by Anding Luo <aluo@uwyo.edu>)
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Advances in sequencing technology have greatly increased the availability of genomic sequences for most model organisms including maize. To leverage these sequences and advance functional genomics in maize, we developed over 100 fluorescent protein (FP) translational fusion lines. These visual makers for nearly all cell compartments permit researchers to address significant questions using cell biology as an interrogative tool. There remain, however, significant bottlenecks in bridging the gap between genome sequence and gene/protein function. Tools for experimental study are particularly needed and we have now developed an LhG4 two-component transactivation system for experimental use. In this system, promoters with cell or tissue-specific expression activate fthe LhG4 transcription factor in driver lines, which in turn transactivate genes-of-interest driven by the pOp promoter in responder lines. Currently, 34 driver constructs are available to drive expression in shoot and inflorescence meristems, leaves or roots using tissue-specific promoters. Eight responder constructs are currently being analyzed, including *Zea mays* FON2-LIKE CLE PROTEIN1 (ZmFCP1), FLOWERING LOCUS T like, *Zea mays* CENTRORADIALIS 8 (ZCN8), the EMBRYO-SURROUNDING REGION 2C1 (ESR2C1), a *Zea mays* CLE peptide and MALE STERILE CONVERTED ANTHER 1 (MSCA1), a maize glutaredoxin involved in the establishment of phyllotaxy, several proteins that expressed in specific stages of cell division, LIGULELESS1 and LIGULELESS2 among others. Transactivation has been successful using this system: for example, the ZCN8 responder driven by the constitutive promoter driver from maize ELONGATION FACTOR alpha1 phenocopies the early flowering phenotype of ZCN8 overexpression. Currently we are using the constitutive, leaf-specific and/or vasculature promoter drivers to analyze the effect of ectopically expressing ZmFCP1, ESR2c1 and MSCA1. We are also advancing recombineering methods to increase specificity of regulatory elements associated with specific promoters and also testing the efficacy of the 2A protein. We are re-making the LIGULELESS1 and LIGULELESS2 FP fusion constructs through BAC recombineering and testing the efficacy of the different 2A proteins in maize using PIP2-1-CFP construct. All resources are publicly available at the project website at http://maize.jcvi.org/cellgenomics/index.shtml. We encourage new requests for driver or responder lines from the maize community. Contact Dave Jackson or Anne Sylvester for if you have suggestions or need more information

Funding acknowledgement: National Science Foundation (NSF)

**P187**

## Maize embryo morphogenesis: a mutational analysis

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The maize zygote normally develops over approximately 45-days into a mature embryo comprised of five or six leaf primordia and several root primordia. The developing embryo passes through the proembryo, transition, coleoptilar, and stage1 (first leaf primordium) morphogenetic stages followed by the iterative formation of additional leaf primordia during stages 2-6 according to Abbe and Stein (1954). Using EMS-treatment of maize pollen we have produced more than 50 lethal embryo mutants that have no obvious effects on endosperm development except for some reduction in kernel size in some cases. Our dissections to date of mutant embryos in mature kernels show that a majority of the mutations are blocked from the late proembryo through the late transition stage, when the embryo is regionalized into suspensor and embryo proper. During this period the embryo shifts its axis of symmetry, and the internal precursor region of the shoot apical meristem becomes evident in sectioned material. We have located four mutations to chromosome arm 1S among 14 tested using B-A translocation stocks. We will present results of complementation tests between morphologically similar embryo mutants. Germination tests of 25 kernel samples containing mutant embryos revealed that almost all had zero germination or only a few which are likely misidentified kernels. Our earlier work with putative Mutator-induced embryo mutations identified a larger proportion of mutations that were blocked in the coleoptilar and later stages of development (Clark and Sheridan 1991, Sheridan and Clark 1993). The abundance of EMS-induced mutations blocked early in embryo development suggests an abundance of genes acting during this period to regulate the changing patterns of signaling molecules that underlie the cellular changes occurring during the proembryo and transition stages. To address this question we are using confocal microscopy with fusion gene constructs to examine selected gene expression in normal and mutant embryos.

Funding acknowledgement: National Science Foundation (NSF)

**P188**

## Maize *reas1* mutant stimulates ribosome use efficiency and triggers distinct transcriptional and translational responses

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Ribosome biogenesis is a fundamental cellular process in all cells. Impaired ribosome biogenesis causes developmental defects; however, its molecular and cellular basis is not fully understood. We cloned a gene responsible for a maize small seed mutant *dek**, and found it encodes Ribosome export associated 1 (ZmReas1). Reas1 is an AAA-ATPase that controls 60S ribosome export from the nucleus to the cytoplasm after ribosome maturation. *dek** is a weak mutant allele with decreased Reas1 function. In *dek** cells, mature 60S ribosome subunits are reduced in the nucleus and cytoplasm, but the proportion of actively translating polyribosomes in cytosol is significantly increased. Reduced phosphorylation of eIF2α and the increased eEF1α level indicate an enhancement of general translational efficiency in *dek** cells. The mutation also triggers dramatic changes in differentially transcribed genes (DTGs) and differentially translated RNAs (DTRs). Discrepancy was observed between DTGs and DTRs, indicating distinct cellular responses at transcription and translation levels to the stress of defective ribosome processing. DNA replication and nucleosome assembly related gene expression are selectively suppressed at translational level, resulting in inhibited cell growth and proliferation in *dek** cells. This study provides insight into cellular responses due to impaired ribosome biogenesis.

Funding acknowledgement: National Natural Sciences Foundation of China

**P189**

## Microtubule defects in maize katanin mutants

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Asymmetric cell divisions are an important feature in the development of the plant body of vascular plants. One model for asymmetric cell divisions is the division of the subsidiary mother cell during stomatal complex formation in the maize epidermis. A mutant screen for irregularly divided subsidiary mother cells identified the *discordia3* (*dcd3*) mutant. Subsequent cloning efforts determined that both homologues of the p60 subunit of the katanin microtubule severing protein (ktn1a and ktn1b) are disrupted. Here we describe the microtubule defects of dcd3 and an additional ktn1a allele, *Clumped tassel1* (*Clt1*), using immunolocalization of α-tubulin and fluorescent protein tagged tubulin visualized with laser scanning confocal microscopy. Microtubules throughout the cell cycle, including cortical microtubules, preprophase band microtubules, as well as spindle and phragmoplast microtubules all show altered orientation and morphology in the mutants compared to wild type. This illustrates the need for proper microtubule regulation to make coordinated asymmetric divisions.

Funding acknowledgement: National Science Foundation (NSF)

**P190**

## Models of shoot apical meristem morphology for trait mapping in a maize x teosinte backcross population

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The maize shoot apical meristem (SAM) is a small body of tightly regulated pluripotent stem cells, which ultimately generate the entire aboveground plant. Quantitative trait loci (QTL) analysis of three models of SAM size and shape in a biparental maize x teosinte backcross population (MxT Bc2S3) reveal that SAM morphology is a complex quantitative trait. We compared morphology modeling by parabolic estimators, discrete cosine Fourier descriptors (dFDs), and topological descriptor analysis (TDA) for use in QTL mapping. Our results illustrate that increasing phenotypic complexity affects predictions of genetic architecture. Different QTL profiles for each morphological model show that SAM shape and size components can be regulated by distinct, or shared, genetic loci. By combining the predicted effect of detected QTL, we find that the majority of the MxT Bc2S3 SAM morphological space can be traversed by the combinatorial effect of genetic loci, proposing a heritable basis for meristem morphological variation in this population.

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**P191**

## *necrotic upper tips1* is a *VASCULAR-RELATED NAC-DOMAIN (VND)* gene that regulates xylem development and promotes proper water movement during the floral phase

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Maize yields suffer severe declines when water deficits coincide with flowering. In comparison with other growth stages, sufficient water transport during flowering is essential for proper floral organ growth and fertilization. Water stress during the floral phase may result in so-called "tassel blasting" defects: leaf wilting, tassel browning, and sterility. To understand the genetic mechanisms underlying this process, we have identified a mutant from an *Activator (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* stems have difficulty moving water as shown by dye uptake and movement assays. Plastic sections and TEM of *nut1* vasculature showed defective vessel integrity and reduced xylem cell layer diameter, which could provide the basis for its mutant phenotype. The *nut1* mutant is caused by an *Ac* insertion into the transcription unit of a *NAC domain-like* transcription factor. Wildtype revertants were isolated with restored open reading frames caused by *Ac* excision, thus proving the mutant phenotype is in fact caused by the insertion. In addition, an allelic *nut1-ds* mutant was identified with an 8bp insertion causing premature stop codon. Little or no mRNA was detectable in the *nut1-ac* mutant, and no NUT1 protein were detected in both *nut1-ac* and *nut1-ds* mutants by western blot using a NUT1 specific antibody, thus proving that loss of this transcription factor is responsible for the *nut1* phenotype. Based on phylogenetic analysis, *nut1* is closely related to *VASCULAR-RELATED NAC-DOMAIN (VND)* genes that act as master regulators of xylem vessel cell differentiation. The expression domain of NUT1 is spread throughout stem part from the uppermost nodes to the ear nodes, but excluded from the tassel branches and tassel peduncles, constant with a specific roles for water transport after the floral transition. Immunolocalization experiments showed that the protein localizes to developing vasculature, showing that this unique transcription factor functions to maintain xylem vessel integrity during periods of high water movement during the floral phase.

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**P192**

## Optimizing a transient gene expression system using *Zea mays* protoplasts

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Plant protoplasts have been used to assess gene transient expression in many species, and a few protocols have been adapted for use in maize (*Zea mays*). It is particularly interesting to use this system in maize to test transgenes before in planta transformation because of the high cost and large time investment associated with stable maize transformation. With the rapid development of genome-editing tools like TALEN and CRISPR, finding a test system to validate the plasmid constructs before transformation is important, especially for budget conscious labs. Here, we have compared conditions of plant growth for isolation of maize protoplasts for a transient gene expression system. Two growing conditions, light and dark culture, and two tissue types, second leaf and sheath, were tested. After optimization, we achieved successful expression of heterologous genes in protoplasts, including TALENs that can be used for future genome engineering experiments.

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**P193**

## Phenotypic and molecular characterization of heterotrimeric Gγ-subunits in *Zea mays*

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Heterotrimeric G protein signaling controls a wide range of developmental and physiological processes, including cell proliferation and growth. Work of the last decade has shown that G protein signaling in plants does not follow the classical paradigm established in animal systems. Our recent identification and characterization of the maize *COMPACT PLANT2* gene, which encodes the Gα-subunit of the heterotrimeric protein, supports the emerging view, that heterotrimeric G protein signaling is involved in many different processes and likely serves as a hub to integrate different regulatory pathways. Whereas animal genomes have many different heterotrimeric subunits, most plants have only one canonical Gα-, one Gβ-, and up to six Gγ-subunits. Plant Gγ-subunits can be divided into three classes. Type-A Gγ-subunits have an N-terminal coil-coiled γ-domain involved in the interaction with the β-subunit and a C-terminal CaaX prenylation site. Type-B Gγ-subunits have a similar structure, but lack the CaaX motif. The third type-C class consists of an N-terminal coil-coiled γ-domain and a divergent cysteine-rich C-terminal domain. We identified six Gγ-subunits in maize, phylogenitically representing all three classes. Tissue-specific expression will be analyzed by real-time qPCR experiments. To determine their subcellular localization we will generate stably transformed genomic reporter lines that express each subunit tagged with a fluorescent marker in their genomic context. In parallel we will create knockout mutants of each Gγ-subunit using the CRISPR/Cas9 genome editing system. The combination of these complementary approaches will allow to systematically assess heterotrimeric G protein signaling in plants with respect to the organization of plant architecture.

Funding acknowledgement: German Research Foundation (DFG)

**P194**

## PIF1 promotes the degradation of HECATE2 by the E3 ligase activity of COP1 to regulate photomorphogenesis and flower pattern formation in Arabidopsis

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Light signals perceived by photoreceptors play a crucial role in regulating plant growth and development throughout the life cycle of plants. Previous studies have identified many light signaling factors regulating seedling de-etiolation, vegetative and reproductive growth. However, none of these factors have been reported to regulate flower pattern formation. Recently, we have identified a small group of HLH transcription factors called HECATEs (HEC1, 2 and 3) that promote photomorphogenesis by antagonizing the function of PIF1. HECs have previously been shown to regulate female reproductive tract development in Arabidopsis. Here we show that light signaling factors not only regulate photomorphogenesis, but also control flower pattern formation. We observed that *cop1-6* and *pif 1, 3, 4, 5* mutant combinations show over proliferation of stigmatic tissue phenotype similar to that of HEC overexpression plants. The over proliferation of stigmatic tissue phenotype of *cop1-6pif1* is enhanced in the presence of HEC2-GFP overexpression background. Conversely, *hec12* largely suppresses these phenotypes of the *cop1-6pif1*. The E class of flower pattern genes *SEP1* and *SEP3* are oppositely expressed in *pifq* and *hec12* mutants compared to wt. In addition, PIFs directly bind to the G-box region of the promoters of *SEP1* and *SEP3*. *PIF1* and *HEC1, 2* are co-expressed in inflorescence tissues. HEC2 is degraded by the ubi/26S proteasome pathway, and PIFs and COP1 enhanced this degradation *in vivo*. PIF1 and COP1 physically interact with HEC1 and 2. Finally, COP1 can directly poly-ubiquitinate HEC2 *in vitro*, and PIF1 promotes this poly-ubiquitination in vitro consistent with the HEC2 abundance in *pifq* and *cop1* backgrounds both in etiolated seedlings and flowers. In summary, these data show new functions of COP1 and PIFs in regulating flower pattern formation in addition to the negative regulation of photomorphogenesis.

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**P195**

## Plasma membrane proteomics in the maize primary root growth zone: novel insights into root growth adaptation to water stress

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Maintenance of root growth is critical to plant adaptation to drought conditions. Previous work on the maize primary root revealed distinct responses to water stress in different regions of the growth zone: local elongation rates are preferentially maintained in the apical few millimeters, whereas elongation is prematurely inhibited as cells are displaced further from the apex, resulting in a shortened growth zone. These responses involve spatially differential and coordinated regulation of cellular growth processes, including modifications of both cell production and cell elongation. As the interface between the cytoplasm and the apoplast (including the cell wall), the plasma membrane (PM) is likely to play major functions in these processes. In addition, PM proteins may be involved in solute uptake for osmotic adjustment, pH regulation, ion homeostasis and other critical processes in roots growing under water-stressed conditions. Proteomic analyses provide a powerful approach to investigate the physiological basis of stress responses. However, to our knowledge, no proteomics studies have focused on the involvement of PM proteins in the response of root growth to water stress. Using a simplified method for enrichment of PM proteins, we compared the developmental distribution of PM proteins in the growth zone of well-watered and water-stressed maize primary roots. The results identified 432 proteins with differential abundances, and the majority of these changes involved distinct, region-specific patterns of response. The identities of the stress-responsive proteins suggest involvement in diverse biological processes including modification of ion transport and homeostasis, accumulation of sugars and other osmolytes, and changes in membrane lipid and cell wall composition. Integration of these findings with results from previous physiological, transcriptomic and cell wall proteomics studies reveals novel insights into root growth adaptation to water stress.

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**P196**

## Plasmodesmatal transport is regulated by light and the circadian clock

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Plant multicellularity is supported by plasmodesmata (PD), nanoscopic channels in the cell wall that connect the cytoplasm of adjacent cells. PD permit molecules in the cytosol to move between cells, including ions, sugars, phytohormones, small RNAs, and proteins. PD are crucial to plant growth and development, but very little is known about how transport through PD is regulated. To date, PD transport is known to be regulated in response to acute stress or over longer developmental time scales. Here, we show that PD transport fluctuates over shorter time scales, responding to light and the circadian clock.
We conducted a screen for Arabidopsis thaliana mutants defective in regulating PD transport during embryogenesis, and identified two mutants with increased PD transport (ise1 and ise2) that are primarily defective in chloroplast biogenesis. This suggests that signals from the chloroplast regulate PD transport, likely by altering nuclear gene expression. Directly perturbing chloroplast-to-nucleus signaling, such as by interfering with tetrapyrrole biosynthesis or the photosynthetic electron transport chain, is sufficient to alter PD transport. The connection between chloroplasts and PD motivated us to explore whether PD transport is regulated by light signaling pathways. We found that PD transport occurs primarily during the day, with relatively little transport through PD at night. Light is necessary for the increased PD transport during the day, but is not sufficient to stimulate high PD transport at subjective night, suggesting that PD transport is regulated by the circadian clock. Plants growing under shorter day lengths compensate by increasing the rate of PD transport during the day. We are currently using genetic approaches to uncover which light and circadian signaling pathways control the diurnal changes in PD transport. Overall, these findings demonstrate that transport through PD is highly dynamic, adding another dimension of complexity to plant cell-cell signaling.

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**P197**

## PPR151 participates in mitochondrial nad4 intron 1 cis-splicing and seed development in maize

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Splicing of mitochondrial and chloroplast group II introns in plants requires an undefined number of nuclear factors. Pentatricopeptide repeat (PPR) proteins are known to participate in the splicing of introns, but the precise molecular functions of many PPR proteins and their impact on plant development are not completely clear. In this study, we defined the function of PPR151 that is required for mitochondrial nad4 intron 1 splicing in maize. PPR151 is a P-subgroup PPR protein that is targeted to mitochondria. Multiple alleles of the maize ppr151 mutant kernels display a strikingly reduced respiration rate and serious arrest of endosperm and embryo development, giving rise to an empty pericarp phenotype. Null mutation of PPR151 results in deficiency of nad4 intron 1 cis-splicing and dramatically reduced mitochondrial complex I assembly and activity, implying that nad4 encodes an essential membrane NADH dehydrogenase subunit of mitochondrial complex I. The mutation also leads to impairment of other complexes in the respiration chain and enhances the expression of alternative oxidase (AOX). These results indicate that PPR151 is essential to the nad4 intron 1 splicing and mitochondrial oxidative phosphorylation, and the endosperm and embryo development in maize.

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Funding acknowledgement: National Science Foundation (NSF)

**P198**

## Proteome Analysis of Pierce's Disease Tolerant and Susceptible Grape Xylem and Sap

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Pierce's disease (PD) is a significant threat to grape cultivation. The bacterium causing this disease is transmitted by the xylem sap and clogs xylem vessels by the formation of biofilm thus results in plant wilting. Florida hybrid bunch grape are known to be tolerant to PD. The study was carried out to investigate xylem tissue and sap proteome of hybrid bunch grape to better understand the genetic diversity and various metabolic pathways associated with PD tolerance. We include three species to characterize differentially expressed and unique proteins. Comprehensive LC MS/MS approach identified a total of 2519 proteins from xylem and 402 xylem sap proteins. The qualitative analysis of proteins revealed a total 952 xylem proteins are commonly found in all species. Of the 402 proteins from xylem sap, 185 proteins are commonly found in all species. More common proteins (40) are observed between FH and muscadine xylem sap, thus providing further molecular evidence for their PD tolerance in both the species. Xylem tissue and sap share 151 proteins amongst all Vitis species. Among 53 Florida hybrid bunch unique xylem sap proteins, 8 were found in both sap and xylem tissue suggesting that, either these are secreted from xylem vessels or these might constitute as structural and functional proteins. These include peptidyl-prolyl isomerase FKBP12-like, uncharacterized LOC100246012, calreticulin-3-like, probable inactive purple acid phosphatase 1-like, thioredoxin M4, chloroplastic-like, isoflavone reductase-like protein 5, manganese superoxide dismutase, and luminal-binding protein 5-like. Functional analysis revealed that carbohydrate proteins are abundant in bunch grape, while defense related proteins are more abundant in FH and muscadine grape thus conferring their possible role in PD tolerance to Florida hybrid and muscadine cultivars.

Funding acknowledgement: National Science Foundation (NSF)

**P199**

## Proteomic and transcriptomic analysis of *nod* (*narrow odd dwarf*), a maize mutant affected in development

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Maize is a very important agronomic crop and is the most widely grown crop in America. The diversity in maize is high due to the fact it is an outcrossing species. This diversity has been important in breeding unique varieties for each microclimate. Thanks to the sequencing of the maize genome, we are now able to more easily identify the genes that are responsible for the growth and development of the maize plant. In this project we are carrying out characterization of a mutant affected in a number of important traits for maize yield including height plant and leaf angle. The *narrow odd dwarf (nod)* is an EMS mutant, and shows severe pleiotropic defects in vegetative and reproductive organs. The affected gene is an *Arabidopsis thaliana* MCA1 ortholog, it complements a Ca2+ transport defective yeast mutant and it is a plasma membrane-localized protein. Together, these data suggest that NOD is a new developmental regulator in maize that is required for integrating hormonal networks and ion fluxes. To know the pathways within which NOD functions, we are doing identification of NOD protein interactors by immunoprecipitation (IP) using a specific antibody against NOD, followed by LC-MS. As well as RNA-seq analysis comparing wt and nod siblings. Preliminary results from the IP show proteins related to ion transport and hormone signaling, these data will be confirmed using CoIP and BiFC. Analysis of differentially expressed genes from the RNA-seq show overrepresentation of hormone metabolism, signaling, stress and cell wall related genes, which suggests a multifaceted role of NOD in maize developmental processes. All together, the project will help identify the developmental pathways in which NOD is involved, and elucidate its relationship with plant architecture.

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**P200**

## Proteomic profiling suggests control of translation and protein stability is crucial for pollen tube germination in maize (*Zea mays*)

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Germination of the pollen tube in maize occurs rapidly in vitro, with the majority germinating after only 15 minutes. Results from inhibitor studies (e.g., cycloheximide) indicate that de novo translation of polypeptides and protein degradation, rather than activation of transcription, are key facets in the control of pollen tube germination. To test this hypothesis, quantitative profiling of the proteome and phosphoproteome was used to directly assess changes in the transition from mature to germinated pollen. The combined output of three statistical packages in R (IBB, edgeR and PGLEM, FDR of 0.05) identified 393 differentially abundant proteins. Consistent with the inhibitor studies, significant numbers of proteins increase and decrease in abundance (up to ~150-fold) by 30 minutes post-germination. Changes to the proteome were further validated by SDS-PAGE gel silver staining of whole cell protein extracts. Gene ontology analysis revealed several terms associated with translation as significantly enriched among proteins increasing in abundance, further supporting the hypothesis that translation plays a key role in this transition. In addition, 103 proteins are associated with a significant change in phosphorylation state upon germination. These changes are largely independent of change in protein abundance, suggesting that control of protein phosphorylation is also a feature of germination; notably, several kinases and components of the protein degradation machinery show significantly increased phosphorylation. As a genetic test for the biological relevance of the analysis, Ds and Mu insertion mutants with robust PCR genotyping were obtained in 14 genes identified based on the proteomic profiling (e.g, genes that encode proteins that increase in abundance upon germination). Although work is ongoing, both alleles tested to date (a potential transmembrane receptor and a signaling protein) show decreased transmission through the haploid male gametophyte, supporting the idea that this approach identifies cellular components important for pollen function, and possibly pollen tube germination.

Funding acknowledgement: National Science Foundation (NSF)

**P201**

## Receptor like kinase WARTY2 interacts with other RLKs to control leaf epidermal patterning in maize

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Maize leaf epidermal cells are arrayed in a linear pattern established by an expression gradient of developmental signals and their receptors. We previously identified a cell pattern mutant *warty2-1* (*wty2-1*) with overly expanded bulliform-like cells in the adaxial and abaxial epidermis of juvenile and adult leaves. *Wty2* encodes a novel leucine rich repeat receptor-like non-functional kinase (LRR-RLK) in the LRR VII subfamily. To identify WTY2 protein partners, we performed co-immunoprecipitation and mass spectrometry (Co-IP/MS) using a functional WTY2-YFP fusion line. Proteins of membrane extracts from WTY2-YFP and B73 were used in triplicate for Co-IP/MS analysis. The data were analyzed using Perseus software. Significant interactors were determined by a volcano plot-based strategy with the observed protein intensity fold changes between the pull-downs of the WTY2-YFP and B73 plants plotted against their negative logarithmic *t-test* P-values. With False Discovery Rate (FDR) =0.05, S0=5, we identified 136 significant interactors, including 13 LRR-RLKs. With FDR=0.02, S0=5, there are still 62 significant interactors, 8 of which are LRR-RLKs. Other classes of interactors in this category include TUBULINs and Heat shock proteins. The suite of candidates are currently being prioritized and validated.
Through EMS suppressors or enhancers mutagenesis using *wty2-1*, we identified a dominant chimera that shows a normal sector. Sequencing analysis uncovered a premature stop codon mutation in the *wty2-1* mutant gene, Genetic analysis confirmed that the new mutation fully suppressed the original *wty2-1* mutation, which has a Gly to Glu substitution in the binding domain of a putative kinase region. These results suggest that the null *wty2* mutation does not affect patterning of the bulliform-like cells and the original *wty2-1* mutation is dominant negative when *Wty2* is absent. This hypothesis is consistent with analysis of two *wty2* mutants derived from the UniformMu population (*wty2-m1* and *wty2-m2*): in both cases the *wty2-1* phenotype is only visible and mild when either the *wty2-m1* or the *wty2-m2* allele is combined with *wty2-1* allele. Together, these Co-IP and genetic results suggest that WTY2 interacts with other partners, potentially other LRR-RLKs, in transduction of a positional signal specifying differentiation in the maize leaf epidermis.

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**P202**

## Regulation of CENH3 deposition in plants

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The centromere is essential for chromosome segregation in eukaryotes. Centromere identity is epigenetically determined and does not depend on centromere specific sequences. Histone H3 in the centromeric region is partially replaced by a Histone H3 variant, CENH3 (CENP-A in human). The CENH3 nucleosome in the centromere region can recruit other proteins and then form a kinetochore on each chromosome. CENH3 evolves rapidly during evolution, especially in its N terminal region. In human and yeast, previous studies revealed that overexpression of CENP-A would induce ectopic centromeres in chromosome arms, and the CATD (CENP-A centromere targeting domain) is essential for CENP-A deposition. To study the molecular mechanisms regulating CENH3 deposition in plants, constructs for overexpressing CENH3 or expressing modified CENH3 were transformed into maize. We find that in maize: (1) compared to human and yeast, CENH3 overexpression in maize induced centromere expansion rather than ectopic centromere formation; (2) except for the CATD, the last few amino acids in the C terminal of CENH3 are essential for its incorporation into the centromeric nucleosome during centromere propagation.

Funding acknowledgement: National Natural Science Foundation of China

**P203**

## Regulators of proximal-distal patterning in the maize leaf function at lateral organ boundaries throughout the plant

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We have found that genes regulating proximal-distal patterning in the maize leaf play an important role in patterning at other boundaries throughout the maize plant. The reuse of key regulatory factors for boundary creation throughout the plant suggests that dissection of genetic pathways governing boundary differentiation in the maize leaf will provide a platform to compare and contrast regulatory modules governing boundaries in general. We have previously demonstrated that the Liguleless1 (LG1) protein accumulates at the blade-sheath boundary as well as tassel branch-rachis boundaries where it is required for normal branch angle and number. Antibodies were developed against the LG1 regulator, Wavy Auricle in Blade1 (WAB1), and a positive regulator of tassel branch number and ligule differentiation, Liguleless2 (LG2). These proteins accumulate at boundaries within the ear and the tassel suggesting a broader role in boundary creation and differentiation. Because LG2 accumulates early during spikelet meristem differentiation it might function during fate decisions in this tissue. LG2 and WAB1 show partially overlapping accumulation patterns in the ear and tassel and LG1 and WAB1 overlap in tassel branch boundaries. LG1 cannot be detected in the leaves of *lg2-R* mutant plants although LG1 can be detected in *lg2-R* tassel branches suggesting that LG2 may regulate LG1 accumulation in the leaf but not the tassel. Unlike LG1, LG2 does not ectopically accumulate throughout the leaf blade of *wab1* overexpressing plants so despite overlapping accumulation patterns in the tassel and ear, LG2 does not appear to be regulated by WAB1. WAB1 accumulation appears normal in a *lg2-R* background suggesting one is not upstream of the other and that they may converge downstream to regulate LG1. Genomic and systems level analysis will elucidate the regulatory interactions between these proteins while identifying new factors in these important developmental pathways.

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**P204**

## Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport.

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Carbohydrate import into seeds directly determines seed size and must have been increased through domestication. However, evidence for domestication of sugar translocation and the identity of seed filling transporters remained elusive. Maize *ZmSWEET4c*, as opposed to its sucrose-transporting homologs, mediates *trans*-epithelial hexose transport across the basal endosperm transfer layer (BETL), the entry point of nutrients into the seed, and shows signatures indicative of selection during domestication. Mutants of both maize *zmsweet4c* and its rice ortholog *ossweet4* are defective in seed filling, indicating that a lack of hexose transport at the BETL impairs further transfer of sugars imported from the maternal phloem. In both cases *SWEET4* was likely recruited during domestication to enhance sugar import into the endosperm.

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

**P205**

## Spatial and temporal activity of the foxtail millet (*Setaria italica*) seed-specific promoter *pF128*

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The engineering of genetically modified (GM) crops is becoming more and more important, the identification of gene promoters leading to specific expression patterns is crucial for the development of new GM plant generations. *F128* was isolated from a cDNA library of foxtail millet immature seeds. Real-time PCR analysis revealed that *F128* mRNA was specifically expressed in immature and mature seeds. The highest *F128* mRNA level was observed 5 days after pollination and gradually decreased as the seed matured. Sequence analysis suggested that the protein encoded by *F128* is likely a protease inhibitor/seed storage protein/ lipid-transfer protein. The 1,053 bp 5' flanking sequence of *F128* (*pF128*) was isolated and fused to the GUS reporter gene. The spatial and temporal activity of the *F128* promoter was analyzed by stable expression in Arabidopsis, *Zea mays* and *Setaria italica*, and by transient expression in *Setaria italica* and *Zea mays* using promoter-GUS reporter constructs. The *F128* promoter drove GUS expression in the embryo and basal endosperm transfer layers (BETLs) in both maize and foxtail millet. However, the promoter exhibited less specific expression in Arabidopsis, which showed GUS activity in the cotyledons of seedlings. *pF128* activity was higher than that of the constitutive promoter *CaMV35S* and maize seed-specific *19 Zein* promoter. These results indicate that *pF128* is a seed-specific promoter. Its application is expected to be of considerable value in plant genetic engineering.

**P206**

## Studying the function of CLE genes in the evolution of the paired spikelet trait in grasses

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The spikelet is the fundamental unit of grass inflorescence architecture and its number directly affects plant yield. In some grasses, including maize and ~1200 species of the Andropogoneae, along with a subset of the species of the sister groups, Paniceae and Paspaleae, spikelets are produced in pairs, while the remaining grasses such as rice, wheat and switchgrass produce spikelets singly. The genetic basis for this diversity of architecture in spikelet pairing remains unclear. In maize, spikelet pair meristems are enlarged relative to the spikelet meristem, so we hypothesized that the CLAVATA pathway, which regulates meristem size in flowering plants, may regulate their development. Central to the CLAVATA pathway are CLE domain proteins, which are processed to release peptides (the CLE domain) of about 14 amino acids that function as ligands to bind trans-membrane receptors. In this project, we have investigated the molecular evolution of all plant CLE proteins and have grouped them into clusters within which function is putatively conserved. Focusing then on the CLE proteins in meristems, we have used RNA-seq analysis in both maize and Setaria to assess timing of CLE gene expression and to study gene regulation. In addition, we are comparing the genomic context, sequence, expression and function of CLE gene in phylogenetically disparate grasses with either single or paired spikelets. Ultimately, we will translate results from functional studies of CLE genes in maize to other cereal crops and wild grasses. Our research will provide insights in understanding the genetic basis for the derivation of spikelet pairs and the controls of spikelet meristem development.

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**P207**

## *Sympathy for the ligule*: the newly identified phenotypic modifier of *Liguleless narrow*

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Genetic interaction is a dynamic process that can be dependent on many factors such as environment and genetic background. These two factors are particularly important in the case of the maize mutant *Liguleless narrow* (*Lgn-R*) and its newly cloned phenotypic modifier *Sympathy for the ligule* (*Sol*). *Lgn* codes for a previously identified kinase that, when mutated, has pleiotropic developmental defects such as decreased plant height and leaf width and irregular ligule formation. A modifier of this gene, *Sol*, was previously identified in a QTL analysis. Using fine-mapping and EMS mutagenesis, we identified *Sol* as a gene of unknown function that is conserved throughout the grasses. Intriguingly only the version of *Sol* found in certain inbred lines, like Mo17 and NC350, is capable of producing the rescued phenotype. An alternate version of *Sol*, found in inbreds such as B73 and Ms71, cannot rescue *Lgn-R* plants. Evidence indicates that the causative differences between the two versions of *Sol* may be due to amino acid, splicing, and/or regulatory changes. Furthermore the *Lgn-R* phenotype is temperature sensitive. At 23˚C *Lgn-R* plants in B73 show the mutant phenotype, while these same plants die at 33˚C. *Lgn-R* plants in a Mo17 background look almost WT at 23˚C but have a clear mutant phenotype at 33˚C. Further investigations into these two genes should reveal the mechanisms behind these drastic phenotypic differences and hopefully illuminate the dynamics of a unique genetic interaction.

Funding acknowledgement: UC Berkeley

**P208**

## The downstream targets and molecular evolution of the B-class MADS-box genes in *Zea* and the grasses

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The connection between the evolution of the floral MADS-box genes and the evolution of floral diversity has long been hypothesized. However, it has remained unclear how molecular MADS-box changes are translated into phenotypic change. We have revealed a complex history of B-class molecular evolution in the grasses and in the order that contains the grasses, the Poales. We have uncovered a signature of positive selection acting on key amino acid residues that mediate MADS-box protein-protein interactions. Novel MADS-box protein-protein interactions have evolved very recently in the genus *Zea*. In the light of this molecular evolution, we have been working to understand the gene regulatory networks downstream of the maize B-class MADS-box gene, *sterile tassel silky ear1*. Using RNA-Seq and ChIP-Seq, we have begun to reveal how shifting protein-protein interactions alter the transcriptional landscape of floral development.

**P209**

## The maize mutant tassel-less4 has defects in both inflorescence and leaf development

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Zea mays (maize) is important both as an agricultural crop and as a genetic model system. Maize produces a male reproductive structure called a tassel and female reproductive structure called an ear. The tassel produces small, floret-containing branches called spikelets while the ear produces kernels. However, tassel-less (tls) mutants are characterized by an absent or reduced tassel. Eight tls loci have been identified, and two have been cloned. Here, we present the phenotypic characterization and genetic mapping of the tls4 mutant. Morphometric analysis of tls4 mutants shows that tassels produce fewer branches and fewer spikelets than normal siblings. Reduction in spikelet number is due to both a reduction in the length of branches and reduced spikelet density. SEM analysis of immature tassels shows early defects in spikelet pair formation. In addition, tls4 plants exhibit a number of vegetative phenotypes. tls4 produces narrow, rough leaves. Light microscopy of longitudinal sections of adult tls4 leaves shows defects in vasculature patterning. tls4 mutants also exhibit defects in formation of the blade-sheath boundary. Mutant plants are also significantly shorter than normal plants due to both a reduction in internode length and number. Fine mapping using molecular markers indicates that tls4 maps to a 600kb region on chromosome containing 29 genes, including a predicted auxin response factor. Further mapping and sequencing is ongoing to determine the identity of the tls4 mutant gene. Due to its pleiotropic phenotypes, we propose that the tls4 gene plays a critical role as a regulator of multiple stages of plant development.

Funding acknowledgement: National Science Foundation (NSF), American Society of Plant Biologists

**P210**

## The maize transcription factor TEOSINTE BRANCHED1 directly regulates GRASSY TILLERS1 to suppress tiller growth

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Reduced tiller number is one of the most frequently targeted morphological traits selected in modern crops during domestication from their wild progenitors. In maize, two of the best characterized tillering regulators are *teosinte branched1 (tb1)* and *grassy tillers 1 (gt1)* . Genetic analysis showed that these two distinct transcription factors function in the same pathway, with *gt1* acting downstream of *tb1*. However, few direct targets of TB1/GT1 or any other tillering-related transcription factors have been identified in maize. Using TB1 and GT1 specific antibodies, we found that the TB1 and GT1 protein localization domains overlapped in axillary buds, especially in the surrounding immature leaf primordia. Surprisingly, localization was strongly reduced in axillary meristems themselves, consistent with previous RNA *in situ* hybridization results. Interestingly, TB1 binds in vivo to the promoter region of *gt1* through a putative cis-regulatory element containing GGNCCC motifs representing the core consensus binding sequence of TCP proteins. *gt1* mRNA levels are extremely reduced in tiller buds of the null allele *tb1-ref* mutant, consistent with TB1 being an activator of *gt1* expression. The putative TB1 binding site in the *gt1* promoter is conserved in different grasses, suggesting that the regulation of *gt1* by TB1 is part of a common regulatory network operating in several crop plants.

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**P211**

## The PPR-SMR protein PPR91 is required for splicing of mitochondrial group II introns in maize

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The splicing of group II introns is facilitated by a group of cofactors. Pentatricopeptide repeat (PPR) proteins are RNA-binding proteins that involved in RNA metabolism, including intron splicing in mitochondria and chloroplasts. In plant, a small number of PPR proteins acquire a carboxy-terminal Small-MutS-Related (SMR) domain with unknown functions. Here, we analyzed a mitochondrium-localized PPR-SMR protein denoted PPR91 in maize. Null *ppr91* mutants feature abortion of embryo and endosperm development at early stages, and behave as a monogenic recessive trait classified as empty pericarp (*emp*). Analysis of *ppr91* mitochondrial transcripts revealed strong defects in splicing of 12 group II introns, implying that PPR91 is a splicing factor. Immunoblot analysis of core subunits of respiratory chain complexes in ppr91 mutants showed a dramatic reduction in the levels of Nad9, Cox2 and αATPase, but slight increase in Cytc1, indicating the severe defects in the respiratory chain. Using yeast-two-hybrid and BiFC systems, we found that PPR91 interacts with another splicing factor ZmCSF1 directly. ZmCSF1 is a RNA-binding protein localized in mitochondria in maize. Analysis of *zmcsf1* mitochondrial transcripts revealed defects in splicing of 7 group II introns, and 5 of which are overlapped with *ppr91* mutants. All results above indicate that PPR91 is required for the splicing of mitochondrial group II introns via cooperation with ZmCSF1 in maize. However, splicing of other 9 mitochondrial introns behaves normally either in *ppr91* or *zmcsf1* mutants, suggesting that intron splicing involves participation of other factors.

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**P212**

## The regulation of embryo growth rate by Arabidopsis invertase inhibitors

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Sugar plays an important role during plant growth and development. In addition to supporting the metabolic activities, sucrose and glucose are also potent signaling molecules that regulate many aspects of plant development. In most plants, sucrose is produced in photosynthetic tissues and transported via the phloem to storage tissues, such as the roots and seeds. At the site of utilization, sucrose is unloaded from the phloem and hydrolyzed by invertases to glucose and fructose. The activity of invertase largely determines the growth rate of the storage tissues. Since invertases are relatively stable, post-translational regulation by invertase inhibitors is crucial in modulating invertase activity. We have identified two Arabidopsis invertase inhibitors (InvINHs) that are preferentially expressed in the embryo-surrounding region of the endosperm. After endosperm cellularization, InvINHs are down-regulated in a FIS2-dependent manner. FIS2 is a part of the Polycomb Repressive Complex 2 (PRC2) required for endosperm cellularization, which is an important transition during nuclear endosperm development. We hypothesized that PRC2 complex represses InvINHs to increase invertase activity around the embryo, making more hexose available to support the accelerated embryo growth after endosperm cellularization. In support of our hypothesis, embryo growth is delayed in transgenic lines that ectopically express InvINHs in the cellularized endosperm. Our data indicated that the supply of hexose is shifted from the endosperm to the embryo through the PRC2-mediated repression of InvINHs. Since eudicots, such as Arabidopsis, store nutrients in the mature embryo, it is likely that this shift in nutrient supply is less prominent in monocots where the endosperm is the main storage organ. Therefore, data generated through this project could provide the framework for future studies that compare the seed developmental programs between eudicots and monocots.

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**P213**

## The rice zygote transcriptome reveals early initiation of the maternal to zygotic transition and partial activation of the paternal genome before the first zygotic division

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The fusion of the two terminally differentiated gametes, the egg and sperm cells, to form the totipotent zygote is critical to successful reproduction. The growth of the zygote and the early embryo is initially dependent upon maternal factors in the egg cell prior to fertilization. As the embryo develops, it becomes independent of these maternal factors and relies only on embryonically synthesized RNAs and proteins. This process, referred to as the Maternal to Zygotic Transition (MZT), is a landmark event in the life cycle of plants and animals. During this transition, DNA is re-packaged, genes are transcribed, and maternally supplied RNA is degraded. We have investigated the dynamics of this transition in rice by single cell transcriptome analysis of gametes and time-staged zygotes at several time points following fertilization. We find that the zygotic transition initiates by nucleolar fusion, and by late G2, more than 8,000 genes show significant expression changes in the zygote compared to the egg. Over 400 transcription factors are differentially expressed across the time series. To further understand what each parent contributes to zygote development, we examined hybrid zygotes at two time points, karyogamy and late G2, and assessed the proportion of reads coming from each parent using SNPs that vary between the two parental strains. Of the ~16,600 genes with SNP calls, we identified more than 4,000 genes with paternal reads at karyogamy, representing paternal genome activation of approximately 24% of the zygotic transcriptome. However, the bulk of the transcriptome, including genes with de novo expression in the zygote, is still derived from predominately maternal transcripts at both time points studied. We conclude that: 1. Post-fertilization transcription of parental genomes occurs before zygotic division, consistent with initiation of the maternal-to-zygotic transition. 2. The paternal genome shows widespread but incomplete activation compared to the maternal genome.

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**P214**

## The role of CT2 in maize internode development

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Heterotrimeric G proteins are membrane-associated molecular switches involved in the transduction of extracellular signals to induce specific cellular responses by activating downstream effectors. They are composed of the three subunits, Gα, -β and -γ. In contrast to animals, which contain many genes coding for numerous G proteins, most plants contain only one Gα-, one Gβ- and usually three to five Gγ-subunits. We identified COMPACT PLANT2 (CT2) as the maize α-subunit of the heterotrimeric G protein. The phenotype of *ct2* mutants is pleiotropic; they are semi-dwarfed and strongly affected in leaf development, as the mutants produce short, dark green, and more erect leaves. In addition *ct2* shoot meristems are enlarged compared to wild type, demonstrating that CT2 controls cell proliferation and elongation.
Internode elongation is at least in part controlled via brassinosteroid (BR) and gibberellic acid (GA) perception. Our initial analysis of GA response in *ct2* mutants however indicates that *ct2* mutant phenotypes are more related to BR signaling. To assess the possible interaction between CT2 and BR signaling we will analyze *ct2* mutant responses to the application of increasing amounts of BR by monitoring leaf length and width as well as internode elongation. In addition we will analyze the genetic interaction between *ct2* and publically available BR signaling mutants. Furthermore we will pursue a biochemical approach by isolating CT2 interacting proteins in vivo of intercalary meristems using our functional CT2-YFP reporter line in immuno-precipitation experiments. Subsequent mass spectrometry will be performed and identified interacting proteins will be validated by FRET analysis. Future experiments will include engineering loss-of-function mutants of validated interactors using the CRISPR/Cas9 genome editing technique.

Funding acknowledgement: German Research Society (DFG)

**P215**

## The role of *Suppressor of sessile spikelet1* (*Sos1*) in inflorescence development in maize and related grasses

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Meristems control organogenesis in plants in part through the maintenance of groups of undifferentiated stem cells. Upon completion of vegetative development, the shoot apical meristem is converted into the inflorescence (or "flowering branch") meristem. Inflorescences of grass species (Poaceae) bear spikelets, which house the male and female floral organs and are the fundamental units of grass inflorescence architecture. Although solitary spikelets are shared by several major agro-economic crops (e.g., wheat, rice, and barley), paired spikelets are produced by species in at least three grass tribes (e.g., Paniceae, Paspaleae, and Andropogoneae), including maize. Three loci have been identified in the *Suppressor of sessile spikelet* (*Sos*) class of mutants in maize which regulate the production of paired spikelets. Identification of the *Sos* loci will further our understanding of the evolution of the derived paired spikelet trait in maize and other grasses. *Sos1* is a dominant mutant that prevents the formation of the sessile spikelet, causing only single spikelets to form. *Sos1* also has developmental defects in the inflorescence meristem indicating additional roles in development. RNA *in situ* hybridization, genetic interaction analyses, and scanning electron microscopy (SEM) are being used to characterize the defects relative to other maize meristem mutants as well as normal plants. We are also using SEM to determine the developmental ontogeny of selected grasses with single versus paired spikelets in the Paniceae tribe. Our long term goal is to integrate the molecular, genetic, and developmental differences between species with paired or solitary spikelets in order to unravel the mechanisms behind the development of this agronomically important trait.

Funding acknowledgement: National Science Foundation (NSF)

**P216**

## The spatiotemporal patterns of DNA replication and endoreduplication as defined by 3D microscopy of nuclei from developing maize root tip nuclei.

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Spatiotemporal patterns of DNA replication have been described for yeast and many types of cultured animal cells, frequently after cell cycle arrest to aid in synchronization. However, patterns of DNA replication in nuclei from plants or naturally developing organs remain largely uncharacterized. Here we report findings from 3D quantitative analysis of DNA replication and endoreduplication in nuclei from pulse-labeled developing maize root tips (Bass et al., 2014, J Exp Bot 65:2747; Bass et al., 2015, Plant Mol Biol 89:339). In both early and middle S phase nuclei, flow-sorted on the basis of DNA content, replicative labeling was widely distributed across euchromatic regions of the nucleoplasm. Maize nuclei appear to lack the the perinuclear or perinucleolar replicative labeling patterns that are predominantly characteristic of middle S phase in mammals. Instead, the early versus middle S phase patterns in maize could be distinguished cytologically by correlating two quantitative, continuous variables, replicative labeling and DAPI staining. Early S nuclei exhibited widely distributed euchromatic labeling preferentially localized to regions with weak DAPI signals. Middle S nuclei also exhibited widely distributed euchromatic labeling, but the label was preferentially localized to regions with strong DAPI signals. Highly condensed heterochromatin, including knobs, replicated during late S phase as previously reported. Similar spatiotemporal replication patterns were observed for both mitotic and endocycling maize nuclei. These results revealed that *maize euchromatin exists as an intermingled mixture of two components distinguished by their condensation state and replication timing*. These different patterns might reflect a previously described genome organization pattern, with gene islands mostly replicating during early S phase followed by most of the intergenic repetitive regions replicating during middle S phase.

Funding acknowledgement: National Science Foundation (NSF)

**P217**

## The transcriptional co-repressor REL2 is required for maize vegetative and reproductive development

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Plants extensively employ transcriptional repression in order to achieve dynamic and precise control of gene expression for a wide variety of processes ranging from organ development to abiotic stress responses. The maize RAMOSA1 ENHANCER LOCUS2 (REL2) protein belongs to a small family of highly conserved transcriptional co-repressors that are present in mosses and all higher plants, whose founding member is the Arabidopsis TOPLESS (TPL) protein. TPL-type co-repressors do not bind DNA directly, but are recruited by specific transcription factors to inhibit transcription of diverse downstream target genes.
In maize, recessive *rel2* mutants show a variety of background-dependent phenotypes, some with variable expressivity. The ability of REL2 to interact with a wide variety of transcription factors, together with the array of phenotypes observed in *rel2* mutants, suggests that REL2-mediated repression plays a fundamental role in regulating the transcriptional outputs of many pathways throughout maize vegetative and reproductive development. By a combination of genetics, genomics and molecular approaches we are investigating several pathways that use REL2 to control transcriptional outputs. We found that REL2 functions in the regulation of meristem initiation and fate, as well as organogenesis. We will present our results investigating how REL2-mediated repression controls tassel, ear and spikelet formation to better understand the regulatory network that controls the formation of maize inflorescences.

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**P218**

## Tiller development in maize and teosinte

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One of the most dramatic phenotypic differences between maize and its wild ancestor teosinte is that teosinte grows lateral branches from the base of its central stalk. These lateral branches, also referred to as tillers, are common in teosinte but infrequent in maize and absent from most maize inbreds. The maize mutants *teosinte branched1 (tb1)* and *grassy tillers1 (gt1)* produce more tillers than wild type maize. Here, we have analyzed the growth of axillary buds in the wild type maize B73 inbred, as well as *tb1* and *gt1* mutants, and teosinte. In order to understand the growth dynamics of early stage tiller buds, we have analyzed their growth rate in B73 as well as *tb1* and *gt1* mutants, and teosinte. We have found that B73, *tb1*, and *gt1* buds are clearly present at 6 days after planting (DAP), while in teosinte, buds are only obvious beginning at 8DAP. Wild type maize tiller buds become dormant at 12DAP and remain dormant up to 16DAP when we stopped measuring. *tb1* and *gt1* buds continue growing up to our 16DAP measurements. Teosinte buds also continue to grow up to 16DAP, but at a slower rate than *tb1* and *gt1* buds. Our results indicate that B73 buds enter into a dormancy phase, whereas *tb1*, *gt1*, and teosinte buds continue to grow. Furthermore, bud growth in *tb1* and *gt1* mutants is accelerated compared to teosinte. Our data provides a baseline for understanding bud growth, which we can use to time stages for transcript profiling of dormant versus actively growing buds. We anticipate that transcript profiling will help us understand the regulation of tiller bud dormancy and growth at the molecular level, which we hope will ultimately reveal key components of the tiller bud growth regulation network.

Funding acknowledgement: National Science Foundation (NSF)

**P219**

## Transcriptome and Proteome Analysis of Early Stages of Maize Tassel Development

(submitted by Oliver Bear Don't Walk IV <oliverb4@stanford.edu>)
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To determine the temporal progression of RNA and protein expression in immature maize tassels, Agilent microarray transcriptome and mass spectrometry proteome data were collected at 4 stages of tassel development (0.5 cm, 1.0 cm, 1.5 cm, 2.0 cm). Gene expression data utilized four biological replicates and a balanced dye swap design on a two color platform. Proteomics data were generated after precipitation of 100 µg total protein followed tandem mass tag labeling with duplicate samples were digested with trypsin prior to MS/MS peptide sequencing. These 4 stages of growth were chosen because the 0.5 cm and 1.0 cm stages represent tassels samples lacking stamens, the 1.5 cm stage has stamens including anther primordia, and the 2.0 cm stage has anthers as large as 0.1 mm, prior to germinal specification within lobes. Even though gene expression can be a good indicator of cell functions, documenting protein is an even better guide as protein builds most important cell structures and the amount of protein in a specific stage is not always reflected by gene expression at the same stage. From these data, with comparison to dissected anther samples at 0.15 and 0.2 mm, we wish to identify anther-specific early transcripts and proteins and to understand more about immature tassels prior to anther specification.

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**P220**

## Understanding Female Gametophyte Development and Cytoskeleton Interactions in Maize

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Plants have two phases to their life cycle: the diploid phase, or sporophyte and the haploid phase, or gametophyte. The diploid phase ends in meiosis to produce haploid cells. The resulting haploid cells divide to produce a haploid gametophyte, some cells of which differentiate into the gametes. The female gametophyte or embryo sac contains four cell types: the egg cell, synergids, central cell and antipodal which are produced through three mitotic divisions. The egg cell and central cell, the two gametes of the female gametophyte are fertilized by the two sperm cells of the pollen grain to produce the embryo and endosperm of the seed. The underlying mechanism of female gametophyte cell differentiation is poorly understood. Towards this objective, we have characterized a maize female gametophyte mutant *indeterminate gametophyte 2(ig2)*. Mutant embryo sac display defects in nuclei position during the synctium stage of development, extra central cell nuclei and few but large antipodal cells. *ig2* encodes a Microtubule Associated Protein (MAP65).Detailed characterization of this mutant would provide insights into the role cytoskeleton plays in nuclei migration and specification of positional cues in gametophyte development

Funding acknowledgement: National Science Foundation (NSF)

**P221**

## Understanding the function of the *Suppressor of sessile spikelet 2* (*Sos2*) gene in maize reproductive development

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Grasses, such as maize, rice, wheat, and barley, constitute major sources of nutrients necessary to feed the world. These grains are produced from spikelets, the short branches that produce flowers in grasses. A notable difference in spikelet development between grass species is that in wheat and rice, spikelets are produced singly, while members of the Andropogoneae tribe, such as maize, produce spikelets in pairs. In order to better understand this developmental difference, maize mutants that produce only single spikelets are being studied. *Suppressor of sessile spikelet 2* (*Sos2*) is a dominant mutant with defects in meristem development resulting in the production of single instead of paired spikelets in both the ear and the tassel. In the most severe form, *Sos2* produces an ear and tassel with only a few spikelets present. Currently, we are characterizing the genetic and developmental defects in *Sos2* mutants using scanning electron microscopy (SEM) analysis and histology to gain a better understanding of the mechanisms responsible for the double spikelet trait in maize. We are also fine mapping the *Sos2* mutant, which has been narrowed down to three genes on the short arm of chromosome 10. These approaches will provide insights into the function of the *Sos2* gene in the meristem.

Funding acknowledgement: National Science Foundation (NSF)

**P222**

## Understanding the functions of maize heterotrimeric G protein in meristem regulation

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The heterotrimeric G protein complex, containing Gα, Gβ and Gγ subunits, plays important functions in plants, including sugar sensing, response to hormones, stress tolerance and control of development. However, G protein signaling in plants is not well understood compared with that of animals, and their regulatory mechanisms appear to be very different. We have shown that mutants of the maize Gα-subunit *compact plant 2 (ct2)* have fasciated ear phenotypes, resembling the maize CLAVATA receptor mutants (Bommert et al., Nature, 502:7472). Genetic and biochemical studies suggest that the maize G protein α-subunit plays pivotal roles in controlling the shoot apical meristem (SAM) by cooperating with the maize CLAVATA receptor FEA2. Further IP-Mass Spec experiments suggest that another uncharacterized LRR-Receptor like kinase may be also involved in meristem regulation via interacting with both FEA2 and CT2. To further understand the functions of CT2 in meristem regulation, we screened for natural modifiers of ct2 by taking advantage of genetic diversity. We found the *ct2* fasciated ear phenotype was dramatically enhanced in NC350 and HP301 backgrounds, and suppressed in CML69. In the enhanced plants, the ear inflorescence meristems were extremely fasciated and showed multiple branches. By using scanning electron microscope, the enhanced phenotype was detectable at very early stages of ear development (~2 mm ear length). The *ct2* suppressors in the CML69 background only suppressed the fasciated ear phenotype, while other phenotypes, such as dwarf, wide and erect leaves, dense and short spikelets, were not affected, indicating the suppressors may specifically function in ear inflorescence meristem regulation. We are mapping the enhancer and suppressor loci to better understanding the roles of heterotrimeric G proteins in meristem regulation.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P223**

## Unrevealing the genome-wide brassinosteroid network in maize

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Brassinosteroids (BRs) are plant hormones involved in various growth and developmental processes. The BR signaling pathway has been studied in detail in Arabidopsis and its components appear conserved across higher plants. The links between signaling and genome regulation, however, are variable, and such variation contributes to the unique morphological and physiological characteristics of different plants. To better understand the role of BR in maize, we defined the cistrome of the major BR transcription regulator ZmBZR1. We identified 5950 ZmBZR1 targets, approximately 1200 of which are responsive to BR. Comparative analysis suggests that a quarter of the ZmBZR1 targets are conserved between Arabidopsis and maize, many of which are involved in growth regulation and BR synthesis or signaling. Novel findings of the ZmBZR1 network in maize include the enrichment of binding motifs of TCP transcription factors (TFs) involved in lateral branch development and sex differentiation in maize. Further molecular and genetic evidence supports a network of BZR1 and TCP TFs regulating lateral branch development and sex differentiation, which were under selection during the domestication of maize.

Funding acknowledgement: National Institutes of Health (NIH), Department of Energy (DOE)

**P224**

## Using corn seed as a biofactory to produce Manganese Peroxidase

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The goal of the work presented here is to use maize grain as the manufacturing platform for enzymes that have industrial applications. Manufacturing in grain has several advantages including protein stability over many years, being able to meet expanding markets through planting of additional acres rather than building infrastructure, and no need for capital equipment other than planting and harvesting equipment. At 0.1% of dry weight, a ton of enzyme, enough to fill a relatively large industrial market can be produced on 250 acres. Bioremediation of toxic wastes is potentially one of those applications. Manganese peroxidase from *Phanerochaete chrysosporium* was expressed in maize seed using the maize embryo-preferred globulin-1 promoter. The protein is targeted to the apoplast of the cells, primarily the scutellum. An advantage to using a recombinant system is being able to purify a single isozyme in large quantities from the extract. After ammonium sulfate precipitation, the protein is approximately 90% pure after one pass over a Giga-Cap resin. The yield is approximately 1 g pure protein from 2 kg of grain. The protein is 53 kDa in size, has a pH optimum near 5, and a temperature optimum near 25 C. The corn-derived protein efficiently degrades an azo dye, Acid Blue 25, within 30 min to a less toxic compound.

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

**P225**

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

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In recent years, several factors controlling maize inflorescence development were discovered, such as *RAMOSA* (*RA*) genes, which inhibit branching, and *FASCIATED EAR* (*FEA*) genes, which control meristem size and kernel row number. In order to better understand how these genes function and to uncover novel regulators, we used EMS mutagenesis to find enhancer mutations of the classical branching mutant *ra3*, which encodes a a trehalose phosphate phosphatase (TPP), and leveraged the power of natural variation to identify natural modifiers of *ra3* and *fea2*.
We mapped a number of EMS-induced *ra3* enhancer mutations, and found that two independent lines contain mutations in the same enhancer, which is a paralog of *RA3*. We have evidence suggesting that these mutations do not cause a complete loss of function of the protein, but rather impact additional functions besides its enzymatic activity as a TPP.
In a parallel approach, *ra3* and *fea2* (in B73) were crossed to each of the NAM founder lines, and F2s were generated. For both mutants, we identified accessions that greatly enhance the severity of the mutant phenotype. A major *fea2* enhancer locus from NC350 has been mapped using both BSA and screening of F2 populations made using the NAM RILs. Similarly, the *ra3* phenotype is enhanced in the Ki11 background, and we also used BSA and NAM RIL F2s to map underlying loci. This revealed the existence of multiple enhancers and suppressors within the Ki11 genome, which we are now fine mapping.
Combining these approaches, we aim to extend our knowledge of the genetic mechanisms that control inflorescence development. Considering the importance of cereals for food and feed production, modulation of these genes holds great agronomic potential.

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

**P226**

## Using proteomics, network analysis and an enhancer screen to identify new mutants promoting maize subsidiary cell polarity

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Asymmetric division generates two daughter cells of differing fates. Prior to asymmetric division, cells must polarize, setting up the division that leads to different daughters. Therefore, pre-mitotic cell polarization is an essential process leading to correct cell placement and tissue organization. The stomatal complex in maize is composed of 2 guard cells, each flanked by a subsidiary cell. The asymmetric division of the subsidiary mother cell, which yields a large pavement cell and a small subsidiary cell, is a model for plant asymmetric division. Previous studies identified a series of proteins that promote polarization of the subsidiary mother cell. BRK1, a small protein that promotes actin nucleation, polarizes within subsidiary mother cells adjacent to the future guard cell. BRK1 is required for the polarization of two receptor-like proteins, PAN2 and PAN1, which promote polarization of the small GTPase ROP. Finally, actin becomes polarized, and the nucleus migrates towards the future division site. To identify additional components of the polarization pathway, a two-pronged approach was taken. Firstly, multiple proteomics approaches were taken. Co-IP/MS of BRK1, PAN2, and PAN1 was used to identify interactors of these proteins, and comparative proteomics and phosphoproteomics were used to identify proteins and phosphosites with a change in relative abundance in pan mutants. These proteomic data were combined with public *A. thaliana* protein-protein interaction datasets to create a protein network to identify candidate proteins promoting subsidiary cell polarity. Crispr-Cas9 generated mutants in a family of candidates, the *Wpr* genes, were obtained. In a second approach, a *pan2* enhancer screen was undertaken to isolate *enhancer of pan2*, or *enpa* mutants. Thus far, ~50 mutants have been identified and retested. Together, these analyses and mutants generated by forward and reverse genetics will provide tools to further study the mechanism of division polarity in plants.

Funding acknowledgement: National Science Foundation (NSF)

**P227**

## Virtual 3D histology of developing maize seeds using X-ray in-line phase tomography

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Despite increasing demand, imaging the internal structure of plant organs or tissues without the use of transgenic lines expressing florescent proteins remains a challenge. Techniques such as magnetic resonance imaging, optical projection tomography or X-ray absorption tomography have been used with various success, depending on the size and physical properties of the biological material. Here X-ray in-line phase tomography was applied for the imaging of internal structures of maize seeds at early stages of development, when the cells are metabolically fully active and water is the main cell content. This 3D imaging technique with histology-like spatial resolution was demonstrated to reveal the anatomy of seed compartments with unequalled contrast by comparison with X-ray absorption tomography. An associated image processing pipeline allowed to quantitatively segment in 3D the four compartments of the seed (embryo, endosperm, nucellus and pericarp) from 7 to 21 days after pollination.

Funding acknowledgement: European Synchrotron Research Facility (ESRF)

**P228**

## What is fun?

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*Feminised upright narrow (fun1)* is a pleiotropic mutant with sex determination and leaf architecture defects. The tassel is feminised and the adult leaves are narrow and lack auricles giving them an upright habit. Floral development in *fun1* tassels is normal until the pistil abortion phase: the pistil fails to abort ultimately leading to the feminised florets in the tassel. The causative lesion creates a stop codon in a gene conserved in grasses containing a DUF conserved across angiosperms. The identity of the gene has been confirmed by sequencing and non-complementation with a second allele. Immunolocalisation on developing tassels has revealed that the protein localises to developing pedicels and lodicules. The *WAB1:fun1* double mutant displays a synergistic phenotype with a novel leaf structure, implying that these proteins may converge on the same pathway. The *sk1:fun1* double mutant also shows synergism with a novel tassel phenotype. The *d1:fun1* double mutant looks like the *d1* single mutant. Epistasis of *d1* suggests that gibberillic acid may be involved in the *fun1* pathway.

Funding acknowledgement: National Science Foundation (NSF)

**P229**

## *ZmESDP*, a maize endosperm-specific Dof protein gene, regulates aleurone development and starch accumulation in maize endosperm

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The maize endosperm, occupying a large proportion of the kernel, plays an important role in seed development and germination. Current knowledge regarding the regulation of endosperm development is limited. Dof proteins, a family of plant-specific transcription factors, play critical roles in diverse biological processes. In this study, an endosperm-specific Dof protein gene, *ZmESDP*, was identified in maize through genome-wide screening. Expression profile analysis showed that *ZmESDP* is mainly expressed in the endosperm of maize kernel, suggesting that it may contribute to endosperm development. The role of *ZmESDP* in endosperm development was characterized in transgenic maize plants. Suppression of *ZmESDP* resulted in a defective kernel phenotype with a partially patchy aleurone layer and reduced starch accumulation. Further analyses showed that knockdown of *ZmESDP* reduced the expression of *nkd1*, which is involved in aleurone cell differentiation, and that ZmESDP regulated *nkd1* expression by directly binding to the AAAG core element in the *nkd1* promoter. Through genome-wide expression profiling, we found that the starch biosynthesis genes and sucrose synthase genes were down-regulated in *ZmESDP* knockdown kernels. Moreover, ZmESDP was able to directly bind to the *du1* and *su2* promoters. Our study reveals that ZmESDP functions as a positive regulator of *nkd1* in the signaling system controlling aleurone development and plays important roles in starch accumulation during endosperm development.

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**P230**

## ZmSCAFFOLD ATTACHMENT FACTOR B and ZmSCAFFOLD ATTACHMENT FACTOR B-LIKE and their putative role in maize inflorescence meristem development

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The SCAFOLD ATACHMENT FACTOR B (SAFB) has been described in animal system as a RNA Recognition motif (RRM) protein involved in splicing and coupled transcriptional regulation, but its biological or developmental implications in plants are not described yet. In a yeast-two-hybrid screen looking for RAMOSA3 (RA3) physical interactors, ZmSAFB (GRMZM2G031846) came out as one of the stronger candidates, suggesting can be playing a preponderant role in inflorescence meristem branching regulation. The classical mutant ra3 shows increased branching in the inflorescence meristem; RA3 is a trehalose phosphate phosphatase enzyme, expressed encompassing the base of axillary inflorescence meristems. However, the protein is localized in nuclear and cytoplasmic speckles, suggesting additional roles rather than catalyze the trehalose synthesis. Can this specific interaction explain mechanistically how RA3 is working by inhibiting inflorescence meristem branching through transcriptional activation/repression? To explore this hypothesis we also identified a putative homolog to ZmSAFB, called ZmSAFB-L (GRMZM2G152111). In order to characterize further in detail their role and their putative redundancy we are generating CRISPR alleles for both ZmSAFB and ZmSAFBL. Additionally we generated the ZmSAFB-mRFP1, which shows strong constitutive expression in a punctate pattern in nuclear compartments. The strong expression ZmSAFB-mRFP1 lines also showed some developmental defects in leaves, suggesting a broad role for ZmSAFB. These tools will allow us to confirm the RA3 physical interaction in planta and by using cell biology, biochemical and genetic approaches we will determine if ZmSAFB and ZmSAFBL play a role in the RA3 pathway.

Funding acknowledgement: National Science Foundation (NSF)

**P231**

## A cohesin subunit may facilitate centromere pairing in early meiotic prophase in maize

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Homologous chromosome pairing in meiosis is critical for faithful segregation to daughter cells. In most eukaryotes, chromosome pairing is preceded by clustering of telomeres that facilitates the pairing process. We found that centromere association begins at the leptotene stage and is earlier than telomere bouquet formation in maize. Centromere pairing also precedes the pairing of chromosome arms. These observations indicated that centromere association may play an important role in initial homologous chromosome pairing. We also found that the centromere pairing process requires centromere activity because inactive centromeres do not associate at the leptotene stage. Chromatin immunoprecipitation by using CENH3 antibody on meiotic anthers of different stages revealed several proteins by using liquid chromatography mass spectrometry analysis. Structural maintenance of chromosome 3 (SMC3) was identified to interact with CENH3 at the leptotene stage. Immunostaining results showed that SMC3 located on chromosomes and can be detected in the centromeric region of all chromosomes from leptotene to the pachytene stage. SMC3 is dissociated from chromosomes at diakinesis. In the *afd1-1* mutant, which exhibits absence of centromere pairing in early prophase I, SMC3 is not detectable from leptotene to pachytene. In the *phs1* mutant, centromere pairing is incomplete in early meiotic prophase and SMC3 exhibits very weak signals compared to the wild type.

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**P232**

## Asymmetric anaphase segregation of chromosomes in male maize meiosis

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The success of an organism is contingent upon its ability to faithfully pass on its genetic material. Chromosomes must be correctly segregated between dividing cells, a process that is particularly critical in the meiotic divisions that generate an organism's gametes. Two microtubule-based structures essential for accurate segregation are the spindle, which pulls chromosomes apart, and the phragmoplast, which assembles the cell wall between the two new nuclei. Male maize meiocytes lack many of the features that govern the organization and positioning of the spindle and phragmoplast present in other cell types, so we investigated the dynamics of chromosome segregation in this system. We found that the separation of chromosomes in anaphase is not consistently symmetric; the two masses of chromosomes travel unequal distances on the spindle in anaphase. This movement is not due to spindle position; chromosomes are unequally retracted regardless of location of the spindle within the cell. When chromosomes are segregated asymmetrically, the phragmoplast forms equidistant between the nuclei rather than at the spindle midzone as previously thought. This asymmetry in chromosome movement implies a structural difference between the two halves of a bipolar spindle, and may allow meiotic cells to dynamically adapt to metaphase errors or cellular needs.

Funding acknowledgement: National Science Foundation (NSF)

**P233**

## Centromere misdivision, chromosome breakage and pollen irradiation promote de novo centromere formation in maize

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Centromere formation is decided by both genetic and epigenetic factors. The roles of certain centromeric DNA sequences and centromere chromatin environments in centromere assembly in higher plants are poorly understood. De novo centromeres provide a model for studying essential functions of DNA sequences and chromatin states in centromere formation. Here we report eleven de novo formed centromeres from three different processes: the chromosomal breakage-fusion-bridge cycle, centromere misdivision or chromosomal fragments generated by pollen irradiation. These eleven de novo centromeres range in size from 200 kb to more than 2,000 kb, and are located either near the native centromere regions or on the distal regions of chromosome arms. More than twenty de novo centromeres have been documented and the associated DNA sequences have not yet been identified. It seems that de novo centromere formation is common on chromosome fragments in maize and most of the de novo centromeres can transmit stably. De novo centromeres have different DNA compositions from native centromeres, and with the same DNA methylation levels as native centromeres. The changes of chromatin characteristics before and after de novo centromere formation have been studied.

Funding acknowledgement: National Science Foundation (NSF), NSFC

**P234**

## Chromosome dosage effects on the transcriptome

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We used chromosomes resulting from B-A translocations and haploid induction to vary the copy number of the long arm of chromosome 1 in a series of sibling maize plants. This provides the opportunity to model the effects of aneuploidy, equivalent to changing the dosage of all genes on the varied chromosome arm, at the level of gene expression. Previous studies with this aneuploid model have necessarily focused on protein and RNA expression for a small number of genes, but RNA sequencing allows us to determine the effects of massive gene dosage variance on the whole transcriptome. Results to date from leaf tissue suggest several inter-related dosage relationships. Expression levels of many genes located on the varied chromosome have a direct correlation to chromosome dosage. However, a notable subset of the varied genes exhibits dosage compensation, with no expression change regardless of chromosome dosage. There are also expression levels between these limits to varying degrees as well as more extreme outliers. Regarding the rest of the genome, many genes do not show any impact of aneuploidy on their expression. However, among relatively low-expression genes, a substantial subset show an inverse correlation with the varied chromosome *in trans*. A ploidy comparison of haploid and diploid shows more similar profiles. This lends support to the hypothesis that the stoichiometric balance among groups of genes is important. Ongoing work aims to determine which genes are dosage-sensitive, whether this subset is responsive at all dosage levels, which genes on the varied chromosome are responsible for expression changes *in trans* and whether the transcriptome size has changed.

**P235**

## Distinct kinesins on Abnormal Chromosome 10 are candidates for driving neocentromere activity of *knob180* and *TR-1* knobs

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Chromosomal knobs in maize are made up of either of two tandemly repeated sequences, the highly-abundant 180-bp *knob180* and the rarer 360-bp *TR-1*. In the presence of Abnormal Chromosome 10 (Ab10), knobs containing either *knob180* or *TR-1* can act as neocentromeres in that they engage the meiotic spindle and move to spindle poles even though they do not form canonical centromere/kinetochore structures. We identified a gene on Ab10 that is required for neocentromere activity of *knob180* knobs specifically and that encodes a putative C-terminal kinesin. However, we have also identified a second C-terminal kinesin gene that shows an expression pattern and chromosomal location on Ab10 that is consistent with it being the *TR-1* kinesin. Our mutant screens have produced an apparent epiallele and two large deletions alleles of the *knob180* kinesin as well as several more mutants that we are in the process of characterizing by RNA, DNA, and bisulfite sequencing.

Funding acknowledgement: National Science Foundation (NSF)

**P236**

## Dynamic changes of Structural Maintenance of Chromosomes, smc2 and smc4, during the cell cycle of maize

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The structural maintenance of chromosomes (SMC) complexes plays important roles in many aspects of cell cycle, such as chromosome condensation, sister chromatid cohesion, DNA repair, DNA replication and so on. Faithful transmitting of the genetic information of parents requires that interphase chromatin can be condensed into well-defined chromosomes. The chromosome condensing complex is built by a SMC2/SMC4 protein heterodimer and three non-SMC subunits. The study of SMC2/SMC4 of the condensin complex in higher eukaryotic cells has not revealed the dynamic changes of SMC2/SMC4 in plants. To study the function of SMC2/SMC4 during the cell cycle, we cloned the genes of SMC2/SMC4 from maize and produced RNAi transgenic lines of SMC2/SMC4. Using our antibodies against SMC2/SMC4, we observed dynamic changes of SMC2/SMC4 in mitosis and meiosis. The expression analysis of SMC2/SMC4 RNAi transgenic lines showed that SMC2/SMC4 expression level is significantly reduced. We find that in maize: (1) We have cloned the cDNA sequence of SMC2/SMC4. The predicted structures of these two proteins are closely similar to other described SMC proteins. Phylogenetic analysis of these two protein revealed that it belonged to the SMC-type protein family. (2)We produced the antibodies to recognize the SMC2/SMC4 proteins in maize. (3)We confirmed the cell cycle distribution of SMC2/SMC4 by immunostaining and found these two proteins accumulate on the condensed chromatin. In metaphase and early anaphase, the signals of SMC2/SMC4 are more clear throughout the core of the chromatids and we find that there is more intense labeling on the centromeric regions and some strong condensing regions.

Funding acknowledgement: The National Natural Science Foundation of China

**P237**

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

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Fast-Flowering Mini-Maize (FFMM) has been selected to serve as a rapid cycling model within maize itself. FFMM can be routinely harvested 60 days after planting which makes five generations per year easily achievable; with optimized conditions six generations per year may be possible. Because of its short stature FFMM requires less greenhouse space and soil than traditional maize cultivars. Two lines of FFMM have been independently derived by selfing for 11 generations from a hybrid of Neuffer's Early ACR by Alexander's Early Early Synthetic crossed to a selected F2 of Tom Thumb Popcorn by Gaspe Flint. FFMM-A and FFMM-B were selected for fast flowering, rapid seed maturity, compact stature, good pollen yield, and good seed set. A hybrid of FFMM-A and B shows heterosis for height, ear size, and kernel production. Both lines have been Illumina sequenced and FFMM-A has been aligned to the B73 genome. Kernel color markers *R-scm2* and *y1* have been introgressed into FFMM-A and introgression of tissue culture response QTLs for transformation and selection for such is ongoing. The small stature and short generation time of FFMM make it desirable for both research and teaching purposes.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P238**

## Meiotic recombination in synthetic tetraploid lines of maize

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Whole genome duplication, or polyploidy, occurs throughout the evolutionary history of eukaryotes, and is particularly prevalent in plants. A myriad of morphological, genetic and epigenetic changes are induced, both immediately after polyploidization, and continuing over longer timespans in a process known as diploidization. Recombination plays a major part in this process, leading to genome reorganization and, in some cases, adaptation. Evidence exists to suggest that the meiotic recombination pathway itself is impacted by polyploidization, however few details are known. In order to investigate this question, we compared three synthetic tetraploid maize lines, one 'allotetraploid' (Oh43xB73 4n) and two 'autotetraploids' (Oh43 4n and B73 4n), with their diploid progenitors at two main steps of the meiotic recombination pathway. Early in prophase I of meiosis, recombination is initiated by the creation of double-strand breaks (DSBS) in chromosomal DNA. We observed that, on average, the peak number of DSBs in the synthetic tetraploid lines was additive based on the parental lines. Additionally we found a persistence of DSBs later into prophase I in all three synthetic tetraploids, which likely indicates that breaks are being repaired later. Intriguingly, diploid mutants defective in proteins involved in homologous pairing also show this persistence of DSBs. By the end of prophase I, DSBs are repaired, either as crossovers (COs) or non-crossovers. In normal diploid meiosis, COs are formed only between homologous chromosomes. However, in the synthetic tetraploid lines we observed COs between non-homologous chromosomes, sometimes resulting in the formation of multivalents. This also indicates errors in the pairing process. Resolution of these multivalents can result in the large chromosomal rearrangements, and reduced fertility observed in many neopolyploids.

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**P239**

## Mutations within Pseudogenes of Various Ab10 Haplotypes

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Chromosome 10 in maize has an abnormal variant called Ab10. Ab10 is different from N10 because it has a large distal tip on the long arm of the chromosome. The distal tip of Ab10 helps increase it's transmission rate during meiosis greater than 50%. This long arm contains DNA derived from other parts of the maize genome. Three separate isoforms of Ab10 have been identified. These types are based on physical appearance with the location of different markers on the distal tip of the long arm of Ab10. The three types are called type I (Ab10-I), type II (Ab10-II), and type three (Ab10-III). Within these three types of Ab10, there are different haplotypes or varieties that were collected from different geographic regions. In order to identify genetic differences in these haplotypes, we used pseudogenes to check for single nucleotide polymorphisms (SNPs) to help build a phylogenetic tree.

Funding acknowledgement: National Science Foundation (NSF)

**P240**

## Phosphorylation of histone H3 Thr3 by Haspin is correlated with cohesion during the cell cycle

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Haspin-mediated histone H3-threonine 3 (H3T3ph) recruits the chromosome passage complex to the inner centromere during mitosis. However, the significance of H3T3ph in regulating cell cycle is not fully understand in plants. Here, we investigated the distribution pattern of H3-pT3 through cytogenetic observation in maize. H3T3ph signals are distributed along the entire sister chromatids in prophase and drops during anaphase but restricted to the inner parts of functional centromeres in mitosis. In maize meiocytes, H3T3ph is first seen in the pachytene stage and extends to the entire chromosome in metaphase I, but is exclusively limited to the centromere in metaphase II. The distribution pattern indicates H3-pT3 is correlated with sister chromatid cohesion changes during both mitosis and meiosis. Consistently, the H3T3ph disappears in meiosis-specific cohesion mutant afd1. In the sgo1 mutant, the H3T3ph disappears during meiosis II because of the loss of centromeric cohesion protector. In addition, we identify a Haspin kinase in maize (ZmHaspin). The purified ZmHaspin has a role in phosphorylating histone H3-threonine 3 in vitro. Further detailed analysis of the Haspin RNAi transgenic plants will provide new insights to the function of H3T3ph in sister chromatid cohesion.

Funding acknowledgement: National Natural Science Foundation of China

**P241**

## W22 Chromosomes and Chromatin: A Pachytene FISH Karyotype and Genome-wide Differential Nuclease Sensitivity Profile.

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The maize inbred W22 genome was recently sequenced by a consortium of investigators led by T. Brutnell et al. A W22 pachytene FISH karyotype and a nuclease profile of seedling shoot chromatin were made using the same source of W22 (via H Dooner, from Brink's color-converted W22) as that used for sequencing. Cytological maps can assist with genome assembly and accuracy by locating knobs or other repetitive patches refractory to most sequencing strategies. Core chromosomal features (centromeres, knobs, rDNA) were mapped on mid-prophase meiotic chromosomes in centiMcClintock, cMC, units, and compared to previously published somatic W22 karyotypes (colorless-seed W22 inbred released by the Wisconsin AES; Kato et al., 2004 PNAS; Lough et al., 2008 Genetics). For our pachytene FISH experiments, meiosis-stage tassels were initially fixed in Carnoy's solution and stored in 70% ethanol. Anthers with mid-prophase meiocytes were equilibrated in meiocyte Buffer A and fixed with 2% paraformaldehyde prior to microdissection and 3D acrylamide FISH as per Howe et al., (2013; Meth Mol Biol - Meiosis 990:53). Chromosomal positions were measured on computationally straightened pachytene-staged chromosomes imaged by 3D deconvolution microscopy. For a somatic W22 epigenomic annotation, we performed differential nuclease sensitivity (DNS-seq) profiling. The genome-wide mapping of open chromatin with micrococcal nuclease revealed the expected direct correlation with gene activity and with gene density at whole chromosome scale. Together, these additional views of the W22 provide structural and functional frameworks to one of the first non-B73 maize genotype assemblies ideal for genetics, transposon biology, allele-series mutagenesis, epigenetics, and comparative genomics.

Funding acknowledgement: National Science Foundation (NSF)

**P242**

# Framework for a Genome Science Major, Referencing Maize Research for Education

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Genome Science (GS) is a blossoming field underpinning interdisciplinary science with multifaceted societal implications. At the University of Delaware, we are developing an innovative undergraduate curriculum that provides a strong foundational science education, flexible elective domains, platforms for hands-on training and inquiry-based learning. The upper-level curriculum is designed for integrative learning and the opportunity for a +1 Professional or Master's degree via mentored research projects. To help guide the development of the major at UD we surveyed existing GS related programs (fields of genetics and genomics), which suggested a need for transformation in the curricula for genome science education. We also examined acceptance requirements for MD or VMD graduate schools throughout the U.S. and found that an appropriately designed GS program can also provide the preparation needed for future doctors. Here, we present the framework for a GS major including a diagram depicting core domains that reflect the current breadth of GS, subdomains for further programmatic framing, and a concept-map to facilitate course development for the integrated upper-level curricula. We describe how some education components can be designed around new sequencing technologies and using free, open-source software tools. Finally, we identify the Genetics Society of America's Peer Reviewed Educational Portal (GSA PREP) as a place to publish on GS curriculum designs and developments.

Funding acknowledgement: Unidel Foundation Inc.

**P243**

# Genome ENgineering Improvement for Useful plants of a Sustainable agriculture

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World agriculture needs to guarantee food security, replace fossil resources, decrease environmental impact and adapt to global climate change. Whereas France and other European countries presently choose to meet the genetic aspect of these challenges by the sole use of genome breeding, an increasing number of agriculturally important countries enlarge the available gene pool via transgenesis. Despite a certain political reserve transgenesis is already an indispensible technology for French seed companies and public scientists to remain competitive at an international level. Recent scientific advances in the field of transgenesis now provide answers to certain misgivings of citizens and blur the border between breeding and transgenesis. In particular the advent of nuclease technology opens the way to extremely precise modifications of plant genomes at pre-determined sites. In this context it is strategic to reinforce top-level know-how in transgenesis in France, to actively participate in the debate of these new breeding technologies and to demonstrate their applicability in a wide range of crop species. The GENIUS project aims at implementing novel transformation technologies in nine crops (maize, wheat, rice, oilseed rape, tomato, potato, poplar, apple, rose) and the model species Brachypodium.

Funding acknowledgement: ANR

**P244**

## Genome strolling through the *Maize-10-Maze*; a living museum outreach project exhibiting select mutants of maize

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The Maize-10-Maze is a public outreach project organized as a field replicate of the maize karyotype in which 10 individual rows represent the 10 chromosomes of maize. Famous, classical, and interesting mutants will be planted in segregating families, arranged in the same order that they appear on the genetic map. The project is a collaborative FSU-FAMU effort supported by the NSF Plant Genome Research Program, and part of "*Nuclease Profiling as an Integrative Resource for Maize Epigenomics*" (NSF IOS 1444532). Over 100 selected mutants are grown in this chromosome map garden, highlighting classical or recently described maize mutants used by geneticists and breeders and illustrating genetic control of plant growth and development. Three criteria for selecting which mutant to plant - (1) should exhibit a visually striking or cool plant or seed phenotype - such as *Knotted1* (*Kn1*) or *lazy plant1* (*la1*), or (2) should be of agronomic importance - such as *brittle endosperm1* (*bt1*), or (3) should be of major scientific or historic importance - such as *teosinte branched1* (*tb1*). Many of these are naturally-occurring mutants and were discovered more than 50 years ago. We are currently scoring seed stocks for germination and phenotype expression for use in the Summer 2017 *Maize-10-Maze* in Tallahassee, FL. For this conference, we will have clipboards at the poster for soliciting community feedback on suggested additions or updates to the "Cloned?" and "Gene Product" sections of our **Mutant Field Display Placards** (or email bass@bio.fsu.edu). This self-guided public tour of the maize genome will help share the love of maize while promoting education and raising awareness of plant genome research. It will make connections between basic science and society - touching on issues of broad public interest such as food production, plant biology, renewable energy, and genetic diversity.

Funding acknowledgement: National Science Foundation (NSF)

**P245**

## Monsanto Academic Outreach

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Making a balanced meal accessible to all, and doing it in a sustainable way, requires a wide range of ideas and resources. That's why Monsanto has been actively engaging with the academic community to both support scientific efforts as well as build relationships for the future. We highlight outreach activities where we have provided the opportunity to learn more about the broad range of solutions Monsanto is bringing to farmers and the individuals that drive the science as well as fellowships that we have sponsored through our Technology organization. Events have been held across Technology sites including the Mystic, Connecticut site as well as at the global headquarters in St. Louis.

Funding acknowledgement: Monsanto

**P246**

## P3: Predictive Phenomics in Plants, A NSF Research Traineeship Program

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Understanding how particular genetic traits result in given plant characteristics under specific environmental conditions is a core goal of modern biology that will facilitate the efficient development of crops with commercially useful characteristics. Plant characteristics are influenced by genetics and a wide range of environmental factors, including, for example, rainfall, temperature and soil types. Developing methods to effectively integrate these diverse inputs that take advantage of existing biological, statistical, and engineering knowledge will be a key area in this research and training program that will bring together faculty from eight departments. Trainees will engage in cutting-edge research and development areas involving direct data collection and analysis from living plants, including sensor development, high throughput robotic technology, and biological feature extraction through image analysis. This traineeship will use the T-training model to provide students with training across a broad range of disciplines while developing a deep technical expertise in one area. This expertise, in combination with soft skills development, will enable the trainees to work across organizational and cultural boundaries as well as scientific disciplines. To develop understanding of how to share knowledge with diverse groups, the program will provide students with training beyond traditional coursework and research through activities that will develop advanced communication and entrepreneurship skills. Additionally, internship opportunities in industry, national labs, and other settings will equip trainees to choose among the diverse career paths available to scientists and engineers.

Funding acknowledgement: National Science Foundation (NSF)

**P247**

## The International Maize Genetic Resources Advisory Committee (IMGRAC) Meeting at CIMMYT: Implementing a global strategy for the maize community

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Almost ten years ago, in May 2006, a Maize Germplasm Network Meeting was held at CIMMYT Headquarters in Texcoco, Mexico, sponsored by the Crop Trust, the World Bank and CIMMYT. Thirty participants, from Mexican and international institutions, were invited by the meeting co-organizers, Dr. Suketoshi Taba, the Head of the CIMMYT Maize Collection at the time, and Dr. Major Goodman (North Carolina State University, USA), "…to develop and make recommendations on what, where, and how the world maize genetic resources should be conserved in the long term." The end-product of this meeting was a document entitled, "Global Strategy for the Ex situ Conservation and Utilization of Maize Germplasm," which was completed in September 2007. The objective of the document was "…to provide a framework for the efficient and effective ex situ conservation of the globally important collections of maize." In the decade following the completion of the "global strategy document," there have been many advances in the world of crop genetic resources, but the global network vision for maize was never implemented. In 2015, with the support of the Crop Trust, the planning began for a meeting of a newly-formed advisory committee, including representatives from important maize growing areas worldwide, and from public and private sectors, industry and non-profit organizations, with a diversity of backgrounds and interests. The purpose of this meeting was not only to take stock of the areas of accomplishment, but also to address those that have stagnated. We present here the recommendations of the participants and the plan of action for the future.

Funding acknowledgement: The Global Crop Diversity Trust

**P248**

# A comparison between GBS and SNP chip marker systems in molecular profiling of doubled haploid exotic introgression lines in maize

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The Germplasm Enhancement of Maize (GEM) project of the USDA aims to improve maize productivity by enhancing the genetic base of commercial maize cultivars. To accelerate the utility of exotic germplasm in maize breeding, doubled haploid (DH) lines were developed from a single backcross (BC1) generation between landraces from the GEM project (donor parents) and two inbred lines (PHB47, PHZ51) with expired plant variety protection (recurrent parents). A total of 323 and 297 GEM-BC1 DH lines were genotyped using 247,775 genotyping-by-sequencing (GBS) and 7,739 single nucleotide polymorphism (SNP) chip markers, respectively. The mean percentages of recurrent parent genotype (%RP) of the DH lines were 81.4% in GBS and 82.8% in SNP chip markers. The high %RP may be due to the inability to distinguish markers that are monomorphic between exotic and elite parents, as only the elite parents have genotype information. Monomorphic marker correction was implemented based on probability that a double recombination occurred between two flanking donor parent genotypes. After the correction, the mean %RP decreased to 76.5% in GBS and 72.1% in SNP chip markers. Pearson correlation was calculated for %RP in lines that were genotyped by both GBS and SNP markers, and found very strong correlation (r= 0.86) between the two marker systems. Molecular characterization of the GEM-DH lines aims to identify regions of donor parent introgression that could be sources of novel alleles that confer traits of economic importance, and that correction for monomorphic markers would increase the power of detecting associations between SNPs and the trait(s) of interest.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P249**

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

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A multi-institution multi-year collaboration was established to study genotype-by-environment interactions in maize across a diverse set of hybrids and locations. A pilot study conducted in 2014 consisted of nearly 900 hybrids and 25 U.S. locations and one Canadian location. The experiment was highly unbalanced where many hybrids were only tested on a subset of locations, but 10 hybrids were common to all locations. Over 250,000 SNPs were scored on parental inbreds using genotyping-by-sequencing and synthetic genotypes of the hybrids were constructed through the genotypes of the parental inbreds. Data on seven environmental variables (temperature, precipitation, radiation, etc.) recorded every 30 minutes at each location were available. In 2015, this study was also conducted with very small differences with respect to the pilot study.
The objectives of this study were to evaluate the potential of predicting performance at location level for data in (i) 2014 and (ii) 2015 separately; (iii) combining 2104 and 2015 data; and (iv) using 2014 data as prior information for predicting 2015 data. For each one of these objectives (i-iv) four cross-validation schemes were used to construct training-testing relationships according to different real problems that breeders might face in fields:
(1) CV2 - predicting incomplete field trials, some hybrids tested in some locations but not in others (observed locations and observed genotypes).
(2) CV1 - predicting new developed hybrids, materials that have not been observed yet in any trial (observed locations and unobserved genotypes).
(3) CV0 – prediction of relative hybrid performance in entirely new environments, (unobserved locations and observed genotypes).
(4) CV00 - predicting in entirely new environments new developed hybrids (unobserved locations and unobserved genotypes).
For all cases five models were used to perform predictions: (a) L+E, (b) L+E+G, (c) L+E+G+GE, (d) L+E+G+GW and (e) L+E+G+GE+GW where E represents the environmental effect; G the genotype effect; GE the genotype-by-environment interaction effect; and GW the genotype-by-environmental covariate interaction effect. Preliminary results suggest that models accounting for GxE interaction improve predictive ability (10-25%) under CV1 and CV2 schemes; large phenotypic and genotypic datasets provide ample information for good predictions of new environments (CV0); while for CV00 the main source of information was always the genetic component.

Funding acknowledgement: Genomes to Fields Consortium, Iowa Corn

**P250**

## Accelerated development of Quality Protein Popcorn (QPP)

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Quality Protein Maize (QPM), a modified version of the *opaque-2* ( *o2* ) mutant was developed at the International Maize and Wheat Improvement Center (CIMMYT) in the 1980s and 90s. QPM has nearly doubled amount of two essential amino acids, lysine and tryptophan and is considered as a more balanced source of nutrition for humans and monogastric animals compared to normal dent corn. Availability of *o2* in-gene SSR makers has facilitated the conversion of normal endosperm maize to QPM. In this study, we started introgression of *o2* into popcorn by making F1 crosses between several elite popcorn lines and QPM lines. After screening for marker availability, 11 crosses were used for generation advancement. By marker-assisted selection, protein analysis and kernel phenotyping in the breeding process, we aim to: (1) introgress *o2* together with modifiers into popcorn elite lines; (2) maximize recovery of popcorn genome; (3) carry out amino acid profiling and popping analysis for the purpose of Quality Protein Popcorn development. Recent progress and plans in this project will be presented.

Funding acknowledgement: ConAgra foods

**P251**

## Accelerating Commercial Plant Breeding and Product Development Through Genomics Data

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The identification of high quality, trait-associated genetic markers is reliant on recombination, phenotyping and the identification of polymorphic markers in chromosome regions of interest. At Monsanto, trait-linked marker development and deployment for commercially relevant traits is accelerated through the use lab automation as well as development and deployment of next generation genotyping and sequencing technologies, with the goal to identify genetic markers for use in high throughput genotyping assays to enable marker-assisted breeding. We highlight examples of deploying new technologies, leveraging public research, and leveraging multiple genotyping technology platforms to enable integration of large amounts of genomic data to accelerate commercial breeding.

**P252**

## An Unexplored Avenue of *Striga*-Resistance in Maize

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*Striga* is a parasitic plant often deadly to maize and sorghum. Crop yields can drop to near zero in *Striga*-infested areas of Africa and the developing world. The collective effect is a major limitation to productivity. A new contribution to efforts aimed at developing *Striga*-resistant maize is provided by the maize mutant, *zmccd8* (*carotenoid cleavage dioxygenase 8*). This mutation allows little to no production of precursors for strigolactone (SL), a maize hormone sensed by the *Striga* parasite and required for germination of its own dormant seeds. We are 1) determining the degree of resistance to *Striga* seed germination and host attraction offered by the *ccd8* mutation, and 2) testing the concept that a relatively modest yield penalty in *ccd8* maize can be countered without losing *Striga* resistance, by breeding strategies based on hybrid vigor and naturally-occurring genetic modifiers. (This work is funded by NSF-BREAD-EAGER program to K. Koch, D. McCarty and J.-C. Guan)

Funding acknowledgement: National Science Foundation (NSF)

**P253**

## Assessing genetic variability for maize growth related traits and sensitivity to water deficit in phenotyping platform.

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Maize is increasingly subjected to water deficit conditions related to global warming. Maintenance of plant growth and improvement of water use efficiency under these conditions is a key issue for maintaining high yields in rainfed areas. However, phenotyping a large number of genotypes for these traits in field conditions under range of scenarios is not straightforward. Recently, the introduction of novel techniques for high-throughput image-phenotyping allows reproducible measurements of functional traits in hundreds of genotypes growing under semi-controlled conditions.
We have analyzed a panel of 255 hybrids in three different experiments with two water scenarios in the phenotyping platform PhenoArch. Plant growth was characterized from plant images taken 10 to 15 times over the vegetative cycle. Plant transpiration was determined and response curves of leaf expansion rate to soil water status were established. Lines were genotyped with 354K polymorphic SNPs and GWAS analysis was performed over evaluated traits.
Plant growth traits showed a wide range of variability (Leaf area: from 0.13 to 0.54 m2; Plant biovolume: from 117 to 577 g) with high heritabilities along the vegetative phase ($H^2$= 0.60-0.91). Variations in plant volume were closely and positively related to changes in plant evapo-transpiration in all situations. Genotypic variability for water use efficiency ranged from 52.6 to 91.7 g FW/l. Soil water deficit caused a large range of responses over the panel. Most sensitive genotypes stopped leaf expansion at -0.6 MPa, while the less sensitive grew up to -2.5 MPa. These traits were analyzed via genome wide association mapping. This study will allow a better understanding of mechanisms involved in the sensitivity to environmental cues. Because such functional traits are not measurable in field conditions it may give way to interpret GxE interaction observed in field network and to assist breeding for drought-prone environments or conditions associated to climate changes.

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**P254**

## Association analysis of field heat tolerant traits in maize and sorghum

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Heat stress caused by high temperature events significantly reduces grain yield and quality worldwide. Enhancing heat tolerance in crop varieties/hybrid is one of the critical factors required for sustaining agricultural production in the future, especially as temperature extreme events and the trend of climate change escalate. Knowledge on the genetic control of heat tolerance and the association of molecular markers with meaningful heat tolerant traits in crops will greatly enhance the effectiveness of genetic selections and breeding for heat-tolerant varieties. However, little progress has been made in this area. In this study, we applied genome-wide association approach and next generation sequencing technologies in the identification of genetic markers/loci associated with major heat tolerant traits in maize and sorghum. Heat tolerance of maize diversity panels and a core of 256 annotated-individually-mutated sorghum (AIMS) mutant library were evaluated under field conditions for multiple years in Lubbock, TX, where sporadic heat waves occur yearly throughout the growing season. We have identified genetic sources for heat tolerant traits at both vegetative and reproductive stages. GWAS analysis identified SNPs significantly associated with heat tolerance in maize. Associate analysis of 256 sequenced sorghum mutants identified a mutation in a gene that plays an important role in thermotolerance in Arabidopsis. Sorghum mutants containing mutations in the corresponding gene showed strong heat injury phenotype. The results of this study laid a foundation in the identification of molecular markers for MAS of heat tolerance in breeding program, and for elucidation of the genetic and molecular mechanisms underlying heat tolerance in crops.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P255**

## Beyond GWAS: Characterizing Drought Response of Elite Temperate and Tropical Maize using a Systems-Biology Approach

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Drought tolerance is becoming an increasingly high-value trait in maize. Due to the difficulty of performing controlled drought field trials, our current understanding the genetics of drought responses is limited. This is particularly true in the context of testcross hybrids representing the diversity of elite temperate and tropical germplasm. Here, recently expired Plant Variety Protection (ex-PVP) inbred lines and Drought Tolerant Maize for Africa (DTMA) inbred lines were crossed to a common tester, PHP02, and grown over two years in controlled-irrigation drought trials in Arizona. Relevant phenotypes (flowering time, plant and ear height, stay-green, yield, and test weight) were collected under both well-watered and drought conditions. Statistical analyses quantified i) the extent of variation present in temperate vs. tropical material, ii) the heritability of traits observed under these treatments, and iii) the phenotypic and genetic correlations between treatments. Traits were then linked to genetic variation via a Genome-Wide Association Study (GWAS) to investigate the basis of drought-affected phenotypes in the temperate and tropical gene pools. Top GWAS hits were subjected to a Weighted Interaction SNP Hub network analysis to identify biological pathways responding to drought and/or corresponding to hybrid performance. Characterization of temperate and tropical germplasm using genome-wide marker effects revealed untapped genetic potential and target haplotype regions for improving drought tolerance in maize.

Funding acknowledgement: The Howard G. Buffett Foundation

**P256**

## Biomass Dynamics of Long-Ear Genetics

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This research examines biomass accumulation in four hybrids. Two hybrids involving two unselected inbred lines derived from BSLE(M-L)C30 [i.e., Long Ear (LE) genetics] and one short-season inbred line (CG60) and two conventional short-season hybrids. Initial results show that the LE hybrids accumulate more biomass by silking than Conventional Ear (CE) hybrids and that it does not appear to be due to canopy closure differences. The initial results suggest that the BSLE(M-L)C30 population is a useful source of genetic variation to enhance biomass in short-season maize hybrids.

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

**P257**

## Brassinosteroid and Gibberellin control of plant height in a phenotypic-selected introgression library in maize

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Maize plant height (PHT) is a complex trait which has been intensively selected and studied in maize breeding. Previous studies focused on elite germplasm with reduced height or dwarf mutants. There is a need to study PHT increasing alleles desirable for bioenergy maize production. We introgressed dominant PHT but not flowering time increasing alleles from tropical germplasm into elite temperate lines to create two phenotypic-selected introgression libraries, which can be used as pre-breeding materials for future biomass production. Genome-Wide Association Studies (GWAS) were used to dissect the genetic architecture underlying PHT in our library, 73 genomic loci were discovered to be related to tallness, 30 overlapped with previous reported hot spots and 20 co-localized with candidate genes within the Brassinosteriod and Gibberellin pathway. As these two plant hormones are well known to regulate PHT with least negative side effects, we explored their control of PHT at physiological level. With selection of PHT increasing alleles, “stronger” hormone pathway alleles were co-selected into our library, seed harvested from tall individuals show stronger hormone responses than from the short ones within one family. Moreover, we found that seedling stage hormone level can be used to predict mature stage PHT performance for families with high level of heterozygosity, corresponding to previous discoveries that plant hormones are related to maize heterosis. We conclude that our libraries are enriched for favorable PHT-increasing alleles, which offer opportunities for future PHT gene isolation and to breed novel cultivars for biofuel production. The Brassinosteroid and Gibberellin pathway has a profound effect for the regulation of plant height increase and heterosis at the physiological level in maize.

**P258**

# *Brown midrib2*: a role in resistance to Fusarium ear rot and fumonisin contamination

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The fungus *Fusarium verticillioides* causes Fusarium ear rot (FER) and produces the mycotoxin fumonisin in maize, which can result in yield losses and human health risks. Previous studies have suggested that resistances to FER and fumonisin contamination are strongly genetically correlated and associated with overlapping quantitative trait loci (QTL). Traditionally FER is scored based on the percentage of the ear presenting symptoms. However, FER symptomatology is highly variable with respect to fungal pigment accumulation and kernel tissue degradation. To better understand resistance to *F. verticillioides*, we quantified fumonisin content via ELISA and evaluated FER using the traditional method, a qualitative categorization of symptomatology, and a quantitative measure of fungal pigment accumulation in the CML333xB73 family of the nested association mapping population. In contrast to previous studies, the traditional measure of FER was weakly correlated with fumonisin contamination and the two traits did not share QTL. Fumonisin accumulation was significantly associated with symptom category, where more severe symptom types had greater fumonisin content than less severe types. In addition, QTL associated with symptom type colocalized with fumonisin QTL. One of these shared QTL was adjacent to the gene *brown midrib2* (*bm2*), which encodes methylenetetrahydrofolate reductase (MTHFR), a key enzyme involved in methionine biosynthesis. In maize kernels, MTHFR expression is highest early in development and methionine is primarily stored in the endosperm glutelin proteins. Methionine is converted to S-adenosylmethionine (SAM), a precursor of ethylene in maize. Ethylene has been implicated in stimulating fumonisin production in *F. verticillioides*. SAM is also required for biosynthesis of fumonisin and the pigment bikaverin in *F. verticillioides*. We measured methionine content in mature grain as a putative indicator of MTHFR activity and SAM availability. We hypothesized that methionine content would be positively associated with FER severity and fumonisin contamination and that methionine QTL would colocalize with *bm2*.

**P259**

# Characterization of genetic diversity, population structure and linkage disequilibrium within and across 35 European maize landraces using high-density genomic data

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Maize landraces represent a valuable source of genetic variation based on the following considerations: i) Only a small number of landraces served as sources for modern elite germplasm, therefore landraces can be expected to contain unexploited allelic variation. ii) Due to their adaptation to specific local environmental conditions they are likely to harbor unique favorable alleles for biotic and abiotic stress resistance. iii) Because of many generations of random mating, landraces are assumed to have low levels of linkage disequilibrium (LD) and no population substructure, making them ideal for genetic mapping. Up to date, these assumptions could not be appropriately tested as most previously reported diversity statistics and LD levels for maize landraces were based on measurements across landraces with only one individual per accession and /or low-density molecular marker data.
We investigated genetic diversity, population structure and LD in a broad panel of 35 European maize landraces by genotyping 22 to 48 individuals per landrace with 609,442 single nucleotide polymorphism markers. Percentage of polymorphic markers and average nucleotide diversity *pi* indicated high genetic variation within landraces and $F_{st}$ values suggested substantial genetic variation between landraces. Population structure analyses revealed genetic clustering of landraces mainly according to geographical origin and kernel type (Dent/Flint), absence of population substructure within landraces and moderate levels of admixture between landraces. Unlike most previous studies, which reported very low LD levels based on measurements across many landraces with few individuals, we found that within most landraces, LD decay distances ($r2 < 0.2$) were within 100 to 500 kb. Based on this unique and extensive dataset, we present results on the influence of sample size, sample composition and marker density on diversity and LD measurements within and across landraces.

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**P260**

## Combining Ability of New Maize Lines for Yield and Aflatoxin Resistance

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Pre-harvest aflatoxin contamination caused by the fungus *Aspergillus flavus* is a common problem in corn production under the hot and humid conditions of the Southeast USA, and can cause significant economic losses. Genetic resistance to *A. flavus* infection and aflatoxin accumulation is needed to minimize the risk to growers. Seventeen inbred lines from five different pedigrees have been developed in our breeding program with good agronomic traits and good aflatoxin resistance. A line by tester (Design II) crossing experiment was conducted, in which these 17 inbred lines were used as males, each crossed onto six female testers. Three females (NC368, LH195, and LH150) are of the stiff stalk heterotic group, and three (LH210, LH51, and LH132) are considered non-stiff stalk. The hybrids were planted in replicated trials at Tifton, GA in 2014 and again in 2015 to evaluate aflatoxin resistance. All top ears in each plot were inoculated with *A. flavus* isolate NRRL 3357 using the side-needle technique 14 d after silking. Ears were hand-harvested 60 d after silking and dried at 40°C to stop further aflatoxin production. Each plot was shelled and ground in bulk, and aflatoxin quantified using the Vicam Afla-test procedure. Grain yields were measured in separate replicated trials using a two-row plot combine equipped with scales and moisture sensors. Agronomic traits such as ear height, plant height, days to anthesis, days to silking, and lodging were assessed as well. Among the testers LH210 had the highest general combining ability (GCA) for grain yield. In contrast, LH51 had negative GCA for grain yield and unfavorable GCA for aflatoxin accumulation. Five male lines had favorable GCA for aflatoxin resistance. Some of the best specific hybrids, combining high yields with lower aflatoxin and low lodging included LH210 x GT1328, LH195 x GT1309 and LH210 x GT1329.

Funding acknowledgement: United States Department of Agriculture (USDA), Georgia Corn Commission

**P261**

## Conditioning Tests for Selection with Information on Segregation Distortion

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We are studying responsive allelic variation associated with phenotypic change in several populations, including a tropical synthetic (TropicS) population created by systematic inter-mating of seven inbred lines. Examination of selection response through the lens of allele frequency change, which is used to detect signatures of selection, may be biased by segregation distortion. Loci that exhibit distortion can rapidly change in frequency, but may not be important drivers for selection response and may also indirectly alter the way a population responds to selection. We have begun examining genetic properties of F2 populations derived from the initial crosses of the TropicS population. Here, we present results from one cross, CML341 x CML373, for which we constructed a genetic map using imputation-less GBS data for which markers belonging to a single linkage group showed perfect association with a single chromosome of the reference map of B73. On average, about 80% of the markers displayed excess heterozygosity relative to expected levels. Non-random distortion was found in multiple genomic regions, but these did not co-localize with previously mapped gametophytic factors in maize. The methods from this cross will be utilized in the analysis of the remaining three F2 populations to more completely inform analysis of allele frequency change in populations selected from the TropicS base population.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P262**

## Detecting changes in maize root exudate composition after cold stress using NMR and Principal Components Analysis

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Early spring planting is one way for farmers to extend the growing season and increase yield of maize. However, the current commercial maize varieties are still relatively cold sensitive, which makes it necessary to identify genetic loci that would increase cold tolerance. Root exudates are appreciated for their role in mediating below-ground communication and have particular importance in protective responses to biotic and abiotic stress. Researchers have demonstrated that plant root exudates such as proline play a crucial role in mediating abiotic stress. Tracking changes in root exudate composition and quantity could be an important way to measure seedlings' response to cold. Maize has a long breeding history and the species has been adapted for cultivation across a wide range of latitudes. This genetic diversity has been used to create mapping populations, such as the Intermated B73 x Mo17 (IBM) lines. The IBM population has been used in other research to identify genetic loci conferring desirable cold stress responses, often by using gross measurements of above-ground tissue such as shoot height. Measurement of below-ground phenotypes including root exudate composition becomes important due to the necessary role plant root exudates play in protecting plants from abiotic stress. This study aims to detect changes in root exudate profiles collected from maize seedlings after eight cold stress conditions. B73 and Mo17 seedlings were given cold stress for 24 hours at 4°C or 10°C during imbibition, germination, one day after germination or three days after germination. 1H-NMR was used to collect spectra of root exudates, spectra were processed and binned, and Principal Components Analysis was used to detect profile differences based on cold treatment conditions and genotype.

Funding acknowledgement: National Science Foundation (NSF)

**P263**

## Development of a maize MAGIC population and applications in quantitative traits dissection

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Multiparent Advanced Generation Inter-Cross (MAGIC) population is an ideal resource for quantitative traits dissection. Here, we present a modified maize MAGIC population tailor-made for breeders, consist of 1404 progenies derived from 24 Chinese elite inbred lines. Both the progenies and the founders have been sequenced at low coverage (1x and 10x, respectively) and also genotyped by Illumina 200K SNP array. Over 50 million SNPs, 2 million indels and one million structural variants have been characterized under carefully quality control. The genomic data provides a good opportunity for studying the mutation, recombination and selection. Through GWA analysis on many agronomic traits in multiple environments for line per se and hybrids by crossing with two testers, this MAGIC population has been proved to have high power and precision as expected and some known genes and many potentially novel loci have been simultaneously uncovered.

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**P264**

## Development of a phenotypic platform to evaluate kernel processing quality for corn chip production

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The corn chip industry relies on the development of food grade corn (*Zea mays*) hybrids that meet quality specifications necessary for the post-harvest processing chain. Currently, relatively large scale evaluation of hybrids in pilot plants is required to determine which hybrids can be managed through a given processing chain, as the specific traits and parameters necessary for a hybrid to be successful are not well understood. Additionally, there are minimal standardized methods available for evaluating processing traits in hybrids on a small scale. Using a set of six hybrids that have been tested for their ability to be managed in the Frito Lay corn chip processing chain, we evaluated multiple bench top phenotyping methods linked to key steps in the processing chain. Each assay was evaluated to determine its effectiveness as a proxy for kernel processing success. Through this study, we seek to develop a benchtop or small scale phenotyping platform that will aid breeding programs in effectively evaluating and developing food grade corn hybrids that can be successfully managed in a corn chip processing chain.

Funding acknowledgement: PepsiCo Inc

**P265**

## Dissecting the genetic underpinnings of tillering in a sorghum RIL population [*S. bicolor* (Tx7000) × *S. propinquum*]: a preliminary report

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Tillers are vegetative branches that grow at or near the ground level, and they play an important role in plant biomass accumulation and grain yield. In this study, we are using QTL analysis to elucidate the genetic architecture of tillering in sorghum, one of the world's major food and biofuel crops. Tillering is an economically important trait, and understanding the factors that regulate the number, size, and fertility of tillers is of critical importance in optimizing yield potential. Recent studies have pointed to a number of QTLs influencing tillering in sorghum, however, the full suite of genes that comprise the tillering network is unknown. Here, we are applying a combination of traditional genetic and modern genomic approaches to elucidate the genetic tillering network using a recombinant inbred mapping population derived from a cross between a domesticated cultivar with suppressed tillering and a wild highly-tillered relative [*S. bicolor* (Tx7000) × *S. propinquum* (courtesy Bill Rooney, Texas A&M University)]. Preliminary greenhouse phenotyping of F5 RILs grown in restricted pot sizes indicates moderate genotypic effects on tillering. At present, we are collecting additional genotype data via whole genome Illumina sequencing of F5 RILs to increase our number of single nucleotide polymorphic (SNP) and structural variant (SV) markers for genetic mapping. To date, we have sequenced 88 F5 RILs at an average 4x depth and have obtained ca. 1,092 and 68 segregating SNP and indel markers, respectively. Preliminary QTL analyses have indicated multiple markers for tillering consistent with known tillering QTLs; in addition, novel QTLs were discovered on linkage group 2. We will present these preliminary results aimed at delineating the tillering pathway in sorghum.

Funding acknowledgement: National Science Foundation (NSF)

**P266**

## Divergent Selection for Shoot Apical Meristem (SAM) Size

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The maize shoot apical meristem (SAM) is responsible for the development of all aboveground organs in plants. Previous research demonstrated that SAM volume correlates with agronomically important adult plant traits including height to primary ear, days to anthesis, leaf node number, and stem diameter above the primary ear. To further investigate these correlations, we set out to breed two populations of maize divergently selected for large and small SAM size. Direct phenotypic selection for SAM size is difficult because measurement of SAM volume is a destructive assay. Here, we report the use of genomic selection beginning with a diverse panel of 382 maize inbreds to develop divergent populations. Over three cycles of selection we demonstrate a 75% increase and a 47% decrease in average predicted SAM volume for the large SAM and small SAM populations, respectively. After one more cycle of selection, individuals from the large and small SAM populations with the most extreme predicted values will be used for doubled haploid production. These doubled haploids will then be measured for SAM volume, which will measure the efficacy of our genomic selection program, and serve as a resource for further analysis of the relationship between SAM size and adult traits.

Funding acknowledgement: National Science Foundation (NSF)

**P267**

## Dynamic regulatory changes in nitrogen utilization genes from a century of selection for seed protein concentration in maize

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Begun in 1896, the Illinois Long Term Selection Experiment (ILTSE) is the longest running genetic experiment in higher plants, with more than 110 cycles of divergent recurrent selection producing known extremes for grain nitrogen concentration. The ILTSE is a unique resource for maize functional genomics because of its genetic variation for N uptake, utilization, and growth response to N, each of which are influenced by many genes. Based on RNA sequencing of the high and low protein inbred lines, 7% of genes on average are differentially expressed in the leaf, earshoot or seeds. Similarly, only 5% of SNP loci were found to be fixed for different alleles between the high and low protein populations. Meanwhile, surprisingly high levels of allelic diversity remain within the population. Continued response to selection using this standing variation may be an effect of canalization. The asparagine cycling system is an example of a change that allowed cryptic variation to become important for continued response to selection. Two genes involved were tested using near isogenic lines to determine the effect on seed protein. Genes found to be important in the ILTSE population could be utilized to improve nitrogen use efficiency in breeding maize and other crops.

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

**P268**

## Effect of *teosinte parviglumis* introgression into a B73 background on root system architecture under low phosphorus conditions

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Maize was domesticated approximately ~9,000 years ago from *Zea mays ssp. parviglumis*. Subsequently, modern maize was bred for yield traits, typically in optimal field conditions of as fertilization, and water regimes. This process has inadvertently selected for root phenotypes that are not necessarily optimized for agricultural conditions with limited external inputs. Wild relatives of modern crops provide a rich source of genetic diversity that can be mined for traits to improve crops for better performance under low-input and stressful conditions.
In this work, we are characterizing a BC4 near isogenic line (NIL) of B73 carrying ~6 percent *Zea mays ssp. parviglumis* introgression that was initially mapped to a single region in the long arm of chromosome 1. This NIL, that we named Z031E0061, was chosen based on previous meta QTL analysis of loci impacting maize root architecture. Z031E0061 produced more crown roots than B73 when plants were grown under low phosphorus conditions.
We performed an RNA-Seq analysis under both - P and + P conditions for both B73 and Z031E0061, and identified several genes that showed contrasting expression responses with respect to P availability. In addition, the RNA- Seq data help us to identify parviglumis introgression regions at a higher resolution than the previously available marker data. This analysis revealed at least two other introgression regions in chromosomes 2 and 7, besides the previously identified region in chromosome 1. In order to identify which introgressed regions are responsible for the observed low phosphorus adaptation response, we are generating a segregating F2 population using selfed NIL x B73 plants. These plants will be phenotyped for several root and shoot traits to then establish associations between the different introgressed regions and the observed phenotypes.

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**P269**

## Elucidation of the Genetic Landscape of Goss's Bacterial Wilt Resistance Via Genome-wide Association as well as Genome Sequencing of Extremely Phenotypic Bulks

(submitted by Sanzhen Liu <liu3zhen@ksu.edu>)
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Goss's bacterial wilt and blight of maize was first identified in 1969. The causal pathogen is the actinobacterium (Gram positive) bacterium, Clavibacter michiganensis subsp. nebraskensis (CMN). CMN occurs in most of the maize growing areas of the central region of the United States where it can cause great yield losses. Prevalence decreased in the 1980s and 1990s, but during the late 2000s, the disease re-emerged and caused a serious threat to maize production. However, the genetic basis for maize resistance to CMN is unknown. As the first step to elucidate the resistance mechanism, we phenotypically screened approximately 650 maize lines with 3-8 replicates. Using HapMap3 genotyping data, we performed a GWAS analysis and identified three genomic loci that are associated with resistance to CMN. To complement the GWAS result, whole-genome-shotgun (WGS) sequencing was performed on two plant bulks, a resistant bulk (R) and a susceptible bulk (S) sampled from resistant and susceptible maize lines, respectively. WGS sequencing can be used to not only accurately measure allele frequencies but determine copy number variation of genes between two bulks. As a result, one candidate gene under one of the GWAS peaks showing allelic divergence in two bulks was identified. Our expression study also indicated that this gene responded differentially to CMN infection. In addition, two candidate loci, separately containing Rp1 and WAK gene clusters, exhibiting higher copies in the R bulk relative to the S bulk were discovered. In summary, genetic data generated from this study will enable enhanced strategies for the economical and sustainable management of Goss's wilt in maize.

**P270**

## Evaluating Teosinte Alleles for Kernel Composition in Maize

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Teosinte (*Zea mays* ssp. *parviglumis*) is the wild ancestor of modern maize (*Zea mays* ssp. *mays*). Teosinte contains much larger genetic diversity compared to maize inbreds and landraces, but is limited by genetic resources to evaluate its value. A population of teosinte near isogenic lines (Teo-NILs) was previously developed to broaden the resources for genetic diversity of maize, and to discover novel alleles for agronomic and domestication traits. The 961 Teo-NILs were developed by backcrossing ten geographically diverse *parviglumis* accessions into the B73 background. Each Teo-NIL bears an average of 2.4 centimorgan (cM) of chromosomal segments from teosinte genome introgressed into B73. The NILs were grown in two replications in 2009 and 2010 in Columbia, Missouri and Aurora, New York, respectively, and Near Infrared Reflectance Spectroscopy (NIRS) and Nuclear Magnetic Resonance (NMR) calibrations were developed and used to predict total kernel starch, protein and oil content on a dry matter basis in bulk whole grains of Teo-NILs. We conducted a joint-linkage quantitative trait locus (QTL) mapping study of total kernel starch, protein and oil content using a genetic map based on the Nested Association Mapping (NAM) population, using 728 polymorphic SNP markers for all three traits. Joint stepwise regression identified two starch, three protein and six oil QTL, which collectively explained 18%, 23% and 45% of the total variation, respectively. A range of strong additive allelic effects for kernel starch, protein and oil content were identified relative to the B73 allele. Our results strongly support our hypothesis that teosinte harbors stronger alleles for kernel composition traits than maize, and can be exploited for the improvement of kernel traits in modern maize germplasm.

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

**P271**

## Evaluating the impact of heterozygosity on nitrogen use efficiency traits in maize

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Increased nitrogen use efficiency (NUE) has been an important target for past maize improvement, with higher NUE enhancing economic and environmental sustainability of maize cropping systems. Extensive field testing, combined with functional genomic analysis, has identified a complex genetic architecture controlling N utilization. Among many quantitative trait loci (QTL), nine robust intervals were determined to account for 5-15% of variation for multiple N utilization traits across years. Additionally, single nucleotide polymorphism (SNP) markers within those regions, and in more than 400 maize genes enriched for regulatory functions and control of N utilization, showed divergent selection among the major germplasm groups for temperate maize production, indicating that heterozygosity is a preferred breeding goal to maximize N utilization and yield. In order to test the impact of heterozygosity on NUE in recent germplasm, pseudo hybrid genotypes were created from genome-wide SNP data for recent elite inbred lines. The hybrid genotypes were scored for heterozygosity across the genome. Three new subpopulations were created that maximized heterozygosity across the entire genome, within the nine QTL regions identified as important for N utilization, or for SNPs that were highly divergent within the mapping populations (Fst value greater than 0.3). As controls, additional selections were made that maximized homozygosity for the same three SNP groupings. The populations were grown in replicated field trials under high and low nitrogen conditions during 2015 and evaluated for grain yield and nitrogen utilization efficiency (NUtE) traits. Initial results from these experiments will be presented.

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

**P272**

## Evaluation of Setaria viridis germplasm for herbicide resistance

(submitted by Sailaja Maddali <saili71@uga.edu>)
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Herbicide resistant weeds have been reported in 86 crops from 66 countries. We may lose herbicide options for several crops, which will have major economic and environmental consequences for agriculture. Identification of candidate genes and mutations that confer herbicide resistance will help us understand the evolution and transmission of herbicide resistance in weed plants and possibly help us devise new methods to control their spread. In the current study, we analyzed herbicide resistance in green foxtail (Setaria viridis) one of the world's most widespread plants. We have screened 216 accessions of S. viridis collected from all over the world, but with an emphasis on North American accessions. The plants were tested for resistance to fluazifop, glufosinate, glyphosate, nicosulfuron and sethoxydim. Many accessions showed resistance to inhibitors of ACCase (fluazifop and sethoxydim) and ALS (nicosulfuron). We also found four lines showing resistance to glufosinate, an herbicide that inhibits glutamine synthetase, and three lines that showed resistance to the EPSPS-inhibitor glyphosate. We are genotyping these lines either at known herbicide resistance loci or by whole genome sequencing to study the molecular natures and origins/transmission of these herbicide resistances.

Funding acknowledgement: National Science Foundation (NSF)

**P273**

## Expanding the Wisconsin diversity panel to improve GWAS in maize

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Genome wide association studies (GWAS) are a powerful approach to explore the connection between sequence variation and important agronomic traits. To maximize the power of GWAS, association panels are constructed to capture a high level of genetic diversity. The objective of this study was to expand an existing diverse panel of inbred lines for GWAS. A total of 334 inbred lines including elite, exotic, and breeding lines were selected and added to our previous association panel. The entire Wisconsin Diversity population (WiDiv 2.0) consists of 837 inbred lines adapted to the upper Midwest region of the United States. The WiDiv 2.0 population was genotyped with 430,947 RNA-Seq based single nucleotide polymorphism (SNP) markers. Admixture analysis assigned the 837 genotypes to seven subpopulations: stiff stalk (227 inbreds), non-stiff stalk (270 inbreds), Iodent (63 inbreds), sweet corn (35 inbreds), popcorn (15 inbreds), tropical (84 inbreds), and mixed (143 inbreds). The utility of the diversity panel was tested by GWAS of maize cob color. A significant association ($P$ = 9.30E-44) was detected within a 30 kb region of the cob color candidate gene (*p1* gene). The WiDiv population combined with the genomic data provided in this study is a foundation for detecting genomic regions underlying important agronomic traits in maize.This text is not italicized.

Funding acknowledgement: Department of Energy (DOE)

**P274**

## Exploring the genetic basis of maize drought tolerance by genome-wide association study and linkage mapping

(submitted by Guoying Wang <wangguoying@caas.cn>)
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Drought is one of the most important environmental factors influencing maize production in China. Hear we reported the genetic analysis of maize drought tolerance at seedling and flowering stages. One association panel including 368 inbred lines and one double-haploid line population were used for the analysis. The results showed that 185 loci were involved in maize drought tolerance, which were distributed in all of the ten chromosomes. Some of the loci controlling drought toleerance at seedling stage also contriuted to the drought tolerance at flowering stage. We compared the drought stress-related loci by genome-wide association study and linkage mapping and found a lot of overlapped loci. Three loci were confirmed by several F2:3 population. This research will be beneficial to the maize molecular breeding for drought tolerance and cloning maize genes responsive to drought stress.

Funding acknowledgement: The National Natural Science Foundation of China (31330056)

**P275**

## Exposing the hidden half: What can we learn from high-throughput root imaging techniques?

(submitted by Adam Bray <abray@danforthcenter.org>)
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Abiotic stresses including lack of water and nutrients negatively impact crop yields. In the soil, root systems provide plants access to these vital resources, which are often ephemeral and unevenly distributed. Thus, the root system architecture (RSA) -- when and where a plant places its roots -- is key for stress resistance and plant health. Despite their importance to the plant, roots are hidden from view making them difficult to study. Recent advances in high-throughput root phenotyping allow for data collection on RSA in both controlled environments and natural field environments. Combining information from both environments provides new and better understanding of RSA and the environmental factors that influence it. RSA is a highly complex, quantitative trait, and little is known about the specific genes that control it. The secrets hidden below ground will begin to unravel by tapping into the wealth of genetic diversity and resources available in maize. In order to understand developmental changes in RSA, the NAM founders were grown in a controlled greenhouse environment in tree pots with clay pellets. Roots were harvested at 4 weeks, 6 weeks, and tasseling (6-12 weeks), washed, and imaged for analysis using Digital Imaging of Root Traits (DIRT) on iPlant. A duplicate experiment was planted at the Genetics Farm at the University of Missouri-Columbia. Data from the two environments were analyzed and compared to better understand what root traits change over the course of development. Follow up experiments will include screening mapping populations, a dryland breeding population, and mutant lines to identify the genes underlying maize RSA.

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**P276**

## Expression in 7 tissues from 300 lines reveals functional regulatory variants (eQTL)

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In order to find functional regulatory variants from among the tens of millions of SNPs in maize, we are mapping QTL that control expression (eQTL). Gene expression values from 7 tissues in ~300 maize taxa were obtained from a rapid (96/day) low cost (<$15/sample) highly multiplexable reduced representation RNAseq method which I have automated on a robot. Using these expression values, ~100k GWAS mapping experiments were performed per tissue to find regulators of expression for B73 transcripts and de-novo assembled transcripts which are absent from the reference genome. In each tissue, approximately 20% of genes have significant cis regulatory element. Together, these eQTL are used to predict phenotypes and their capacity to explain phenotypic variance is evaluated using a mixed-model variance partitioning approach. Given that single gene cloning and GWAS experiments indicate that natural phenotypic diversity is frequently controlled by intergenic variants which regulate expression, we believe these regulatory SNPs are uniquely positioned to explain natural phenotypic diversity and thus improve genomic prediction in maize.

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

**P277**

## Fall armyworm herbivory affects silica accumulation in corn and rice

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

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

**P278**

# Field based phenotyping using unmanned aerial vehicles (UAVs) and ground vehicles, what are we measuring and what have we learned?

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Methods to collect additional phenotypes, faster, are needed for plant genetic experiments to leverage improvements in genomics, to gain new insights into plant physiology, and to breed more productive crops with enhanced stress resistance. In 2015 the Texas A&M system launched a comprehensive system-wide initiative between field researchers, engineering, and geography to develop unmanned aerial vehicles (UAVs) for use in field-based high-throughput phenotyping (FBHTP) and precision agriculture utilizing a variety of platforms and sensors. The primary objective was to develop a pipeline and team to collect and use this data for answering diverse crop and researcher specific biological and engineering questions. A specific question of interest to maize and sorghum researchers was the estimation of plant height, comparisons with traditional collection methods, and repeatability in the field. Fixed wing aircrafts flying at 120m had medium image resolution but covered larger field plots regularly. Rotary wing aircraft flying at 15m had high image resolution and provided better detailed information for small plots but were collected less often; the best data set contained 1065 yield trial plots with 363 hybrids. Initial raw correlations with height across the entire dataset were positive and significant, but low; manual inspection and improved algorithms improved the correlation and variance component results substantially. Major outcomes of this project have been to identify and develop solutions for the FBHTP pipeline including selection of vehicles, selection of sensors, handling of data, and automated geo-referencing and data extraction; among the largest successes has been developing a strong interdisciplinary team. In 2016 these studies will continue and be complemented by a novel ground phenotyping vehicle that can clear mature corn without damaging the plants.

Funding acknowledgement: United States Department of Agriculture (USDA), Texas A&M AgriLife Research, Texas A&M Engineering Experiment Station, GEOSAT, Texas Corn Producers Board

**P279**

## Finding the Haploid Needle in a Hybrid Haystack: Discrimination of Haploid Maize Kernels by Single Kernel Near-Infrared Spectroscopy

(submitted by Jeffery Gustin <jgustin@ufl.edu>)
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Doubled haploids (DHs) are used widely in commercial and public breeding programs to efficiently advance inbred development. Using DHs, inbred lines can be created in two generations while conventional inbred creation using self-pollination requires 6 to 10. Reducing breeding cycles confers a tremendous advantage on DH technology. DHs are produced by crossing a target genotype with a haploid inducer line to generate haploid seed. Identification of the relatively rare haploid individuals relies on dominant color markers that are expressed in the hybrid. Several inherent drawbacks to the color marker strategy make alternative haploid discrimination methods attractive. For example, haploid kernels cannot be selected in germplasm with suppressed color markers. Near-Infrared Spectroscopy (NIRS) is a non-invasive technology that can potentially be used for efficient haploid identification. Haploid kernels are known to reduce oil content and seed weight relative to hybrids, and oil, weight, and other composition traits can be predicted using NIRS. We tested the ability of single-kernel NIRS (skNIR) to accurately discriminate kernels into haploid and diploid classes. Visually selected haploid and diploid seed from 28 genotypes crossed by a common inducer line were used to calibrate a Partial Least Squares Linear Discriminant Analysis (PLS-LDA) model. A general model built from NIR data from all genotypes classified haploid seed with 83% accuracy. Classification of individual genotypes within the general model ranged from 100% to 33% accuracy. Consistent with prior observations, kernels with haploid embryos had reduced kernel weight and oil content with no change in total starch content. These data suggest that skNIR is discriminating on compositional differences other than anthocyanin accumulation in the embryo. These data also suggest skNIR could be used to identify haploid seeds from inducers lacking anthocyanin markers. In support of this hypothesis, haploids from a color suppressed genotype were classified correctly 70% of the time.

Funding acknowledgement: National Science Foundation (NSF)

**P280**

## Fine-mapping a major maize domestication QTL for ear diameter

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Maize ears are much larger in diameter and have more rows of grain than maize's ancestor, teosinte. This significant difference in ear structure makes it an essential trait to study to better understand domestication. Previously, our lab mapped domestication quantitative trait loci (QTL) in a set of maize-teosinte hybrid recombinant inbred lines (RILs) and identified a large effect QTL on the short arm of chromosome 5 for ear diameter. This QTL co-localized with a large effect QTL for kernel row number, and represents a likely domestication target. Following this work, an unsuccessful effort was made to identify the gene underlying this QTL by fine-mapping with a $BC_2S_3$ heterogenous inbred family (HIF) derived from the RILs. Our lab also mapped this QTL using an independent set of a maize-teosinte $BC_6S_6$ lines segregating for this QTL region. With this set of lines, the QTL was confirmed and more narrowly mapped to a ~2.654 Mbp region. We are now attempting to fine-map the QTL using three of the $BC_6S_6$ lines to create two families that segregate for the QTL. In these two families, the QTL is located between two marker loci which includes 54 candidate genes. We used these markers to identify recombination events in the region and generated a set of 170 recombinant chromosome nearly isogenic lines (RCNILs). More markers within the 4.81 cM region are being identified to fine-map where the breakpoints are in each of the RCNILs. Once the phenotyping data has been collected in the upcoming summer, we'll be able to identify a causal region or gene that is responsible for part of the difference in ear diameter between maize and teosinte. When the gene is identified, we can identify the causative variant within the gene, and test whether selection during domestication has reduced diversity at this gene.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P281**

# Gene duplication at the 27-kDa γ-zein locus is associated with artificial selection for quality protein maize

(submitted by Yongrui Wu <yrwu@sibs.ac.cn>)
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The major storage proteins in maize seed are nearly lysine-free prolamins called zeins, which contribute little to nutritional quality in terms of the protein assimilation. The opaque2 (o2) mutant synthesizing the decreased amount of zeins has a significantly higher nutrition value, however develops a much softer endosperm texture compared to the wild type. By selecting QTLs, the chalky endosperm of an o2 can be converted to a hard version without affecting its high-lysine trait, yielding the well-known quality protein maize (QPM). The QTLs for endosperm modification are designated as o2 modifiers (Mo2s). Mo2-1 is one of the major QTLs associated to enhanced expression of 27-kDa γ-zein, which is essential for endosperm modification in QPM. Mo2-1 is located in bin 7.02 nearby the locus of 27-kDa γ-zein gene itself. Genetic experiments showed that Mo2-1 increases the 27-kDa γ-zein expression in a dosage-dependant fashion and acts downstream of its transcription factor, prolamine-box binding factor (PBF). GWAS analysis with 492 inbred lines showed there is a major QTL controlling the varied expression of 27-kDa γ-zein in natural population, which happens to fall into the same region as the Mo2-1 locus. Linkage analysis of 27-kDa γ-zein expression in the intermated B73 x Mo17 Synthetic 10 DH population (IBM) also located the QTL in the same region. By map-based cloning and BAC sequencing, Mo2-1 was identified to be a 15.26-kb duplication at the 27-kDa γ-zein locus, in which all genes expression was increased. The duplication occurred before domestication; however, it appears to be so unstable that the deduplication frequently occurred in the natural population, while it can be fixed in QPM by artificial selection. Our discovery provides a useful molecular marker that can be used to accelerate QPM breeding.This text is not italicized. *This text is italicized.* This text is not italicized.This text is not italicized. *This text is italicized.* This text is not italicized.This text is not italicized. *This text is italicized.* This text is not italicized.

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**P282**

## Generating clonal progeny in maize

(submitted by Nina Chumak <nina.chumak@botinst.uzh.ch>)
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Apomixis is asexual reproduction through seed. The production of seeds though apomixis, which generates plants that are genetically identical to the mother plant, has considerable agricultural potential to maintain desired complex genotypes, e.g. those of $F_1$ hybrids, over many generations.
Gametophytic apomixis deviates from sexual development in three major steps: (1) meiosis is circumvented or aborted, leading to the formation of unreduced, unrecombined embryo sacs (apomeiosis); (2) embryogenesis initiates without fertilization of the unreduced egg cell (parthenogenesis); and (3) developmental adaptations enable the formation of functional endosperm. The aim of our research is to identify mutations that mimic the major components of apomixis, and to combine them to engineer apomictically-reproducing maize plants.
In a genetic screen we identified the *n*on-*r*eduction in *f*emale 4 (*nrf4*) mutant, which mimics the first step of apomixis: apomeiosis. Homozygous nrf4 plants produce up to 95% unreduced embryo sacs. Using SAIFF-by sequencing technology, the mutation was mapped to *GRMZM2G148133* on the long arm of chromosome 7, and the identity of *Nrf4* was confirmed by two additional mutant alleles. To identify whether *nrf4* leads to first or second division restitution (FDR vs SDR), we analyzed maintenance of heterozygosity in the progeny of *nrf4* mutant plants in comparison to mother plants using a SNP array that enabled the analysis of 10-20 SNPs on each chromosome. The effect of the *nrf4* mutation turned out to be more complex than expected and leads to both FDR and SDR. Nonetheless, depending on the genetic background of the mother plant, up to 11% of the unreduced female gametes were genetically identical to the mother. To our knowledge this is first evidence that production of clonal individuals through seed is possible in maize.

Funding acknowledgement: DuPont Pioneer Hi-bred, Plant Fellows - Marie Curie Actions

**P283**

## Genetic analysis of doubled haploids derived from the Zea Synthetic population

(submitted by Anna Glowinski <acs5fd@mail.missouri.edu>)
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To determine the contributions of allelic variants throughout the maize genome to fitness for both teosinte (Zea mays ssp. parviglumis) and maize (Zea mays ssp. mays), we are using doubled haploids (DH) derived from the Zea Synthetic. The Zea Synthetic is a randomly mated synthetic breeding population comprised of the NAM founders and 11 geographically diverse parviglumis accessions). Here, we present preliminary findings from a two-year trial where we collected agronomic (flowering, plant/ear height, number of ears) and fitness (total number of progeny and progeny weight) related traits in MO, NC, NY and IA. The DH have been genotyped using genotyping-by-sequencing (GBS). The GBS data, combined with whole genome sequencing (WGS) of the Zea Synthetic founders, will be used in an identity-by-descent analysis in order to determine which genomic regions were selected against during the DH process. Genotypic and phenotypic data will be combined in an association analysis to determine phenotype and genotype relationships.

Funding acknowledgement: National Science Foundation (NSF)

**P284**

## Genetic Analysis of Haploid Fertility in Maize

(submitted by Jiaojiao Ren <renjiaojiao789@sina.com>)
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Doubled haploid (DH) technology has become widely used in maize (Zea mays L.) breeding and research. Haploid genome doubling, include haploid female fertility and male fertility, is a key requisite for DH line production. We screened 20 haploid populations derived from Chinese elite inbred lines for haploid female fertility and haploid male fertility. In order to identify haploid female fertility, firstly haploid ears were pollinated by normal diploid maize pollen, then the proportion of female fertile haploids (FFP) and kernel number (KN) on singe haploid ears were calculated. Haploid male fertility was determined by anther emergence rate (AER), pollen production rate (PPR), anther emergence score (AES) and pollen production score (PPS). The experiment was completed in the summers of 2012 and 2013 at Linze and Shangzhuang. All haploid populations showed a certain degree of female fertility. The FFP ranged from 71-100% and no significant genetic variance was observed. The KN ranged from 2.18-59.87%, and the best population showed a 30 times better KN than the worst one. Significant ($p>0.01$) genetic variance for KN among Chinese elite lines was observed and heritability was 0.93. AER, PPR, AES and PPS ranged from 9.8-89.8%, 4.8-85.5%, 0.02-0.83, and 0.02-0.69, respectively. Significant ($p<0.01$) genetic variance was observed among Chinese elite maize lines, and the heritabilities were estimated from 0.68 to 0.91 in these four traits. Correlation coefficients showed that there is a significant correlation ($p<0.05$) between KN and PPS. Further research is needed to clarify the mechanism leading to male and female fertility in haploid maize.

Funding acknowledgement: National Maize Industrial Technology System (CARS-02-09), National Natural Science Foundation of China (31560392)

**P285**

## Genetic analysis of quantitative disease resistance in maize against two isolates of northern corn leaf blight (*Setosphaeria turcica*)

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The instability of R-gene mediated resistance in crop plants to fungal diseases has necessitated research on quantitative disease resistance (QDR) mechanisms so that durable protection can be achieved. When evaluating mechanisms of resistance, it is important to establish that they are broadly effective against the populations of pathogens. To this end, we have conducted field studies of the interactions between the Intermated B73xMo17 Doubled Haploid lines (IBMDHLs) and the *NY01* and *IA01* isolates of *Setosphaeria turcica,* the causal agent of northern corn leaf blight (NLB). A replicated randomized complete block design was executed in two years in Aurora, NY using 385 IBMDHLs inoculated with *NLB-NY01* and in two years in Ames, IA using 330 IBMDHLs inoculated with *NLB-IA01.* In all four trials, disease severity was scored once per week over the two month period following manual inoculation at the V6-V7 stage. Comparisons across the four trials will be made using the area under disease progress curve (AUDPC) measure of quantitative disease progression. Within isolate-location analysis has shown that ~63% and ~54% of the phenotypic variance in AUDPC is accounted for by host genotype in NY and IA, respectively. Preliminary results from QTL analyses of these trials will be presented in the context of whether or not NLB defense relies on the same sets of genetic polymorphisms.

Funding acknowledgement: National Science Foundation (NSF)

**P286**

## Genetic architecture exploration of rind penetrometer resistance and in vitro dry matter digestion in maize

(submitted by Meng Yujie <mengyujie25@126.com>)
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Stalk lodging-resistance and digestibility were two important stalk traits affecting maize yield and feeding values, respectively. Doubled haploid (DH) lines produced via in vivo haploid induction have become an indispensable tool in maize research. To extend knowledge of genetic architecture of rind penetrometer resistance and in vitro dry matter digestion, two populations with different ploidy were developed, genotyped and evaluated. Two quantitative trait loci (QTL) for rind penetrometer resistance were detected on chromosome 1 both in two populations and had a map distance less than 20 cM. Other two QTL located on chromosome 5 (bins 5.05) and chromosome 2 (bins 2.02) could explain 16.90% and 15.70% of genetic variation in DH and haploid population, respectively. One pair of QTL for in vitro dry matter digestion on chromosome 8 was detected in DH and haploid population, explaining 16.00% and 18.60% of the genetic variation, respectively.Potential candidate genes screened and identified in the four QTL support intervals which associated with rind penetrometer resistance and in vitro dry matter digestion were indirectly or directly involved with cellulose and lignin biosynthesis, which participated in cell wall formation.

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**P287**

## Genetic Architecture of the Maize Inflorescence Shattering Trait

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The domestication of modern cereal crops from wild grasses provides a powerful system for studying rapid phenotypic evolution. Wild grasses shed their seeds at maturity to ensure propagation but this scattering or 'shattering' of seed would have been detrimental to cereal grain harvest by humans. The loss of natural seed dispersal would therefore have been key in domestication. In sorghum, rice, and foxtail millet, this shattering trait has been mapped to the *Sh1* locus which encodes a YABBY transcription factor. Maize shattering QTL track to regions on chromosomes one and five containing Sh1 orthologs (*ZmSh1-1.1* and *ZmSh1-5.1+ZmSh1-5.2*). Thus this project seeks to confirm the genetic architecture of the modern maize non-shattering trait on two levels. First, a QTL fine-mapping experiment will confirm that *ZmSh1-5.1+ZmSh1-5.2* is the causative gene and will determine the nature of the change in the gene which led to non-shattering maize from its shattering ancestor teosinte. Preliminary results using developed recombinant inbred lines suggest the causative region lies in the 5' UTR of *ZmSh1-5.1+ZmSh1-5.2*. Second, this project will examine the interaction of the chromosomes one and five maize genes (*ZmSh1-1* and *ZmSh1-5.1+ZmSh1-5.2*) to observe any additive effects between the two genes. Preliminary results suggest a less-than-additive relationship although further analysis is ongoing.

Funding acknowledgement: National Science Foundation (NSF)

**P288**

# Genetics of hybrid performance in maize: QTL detection for biomass production in a reciprocal multiparental design

(submitted by Alain Charcosset <charcos@moulon.inra.fr>)
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Understanding genetic architecture of hybrid performances is of key importance for allogamous species such as maize (Zea mays L.). We developed two multiparental populations corresponding each to one of the main heterotic groups used for maize silage production in Northern Europe (the dent and flint groups). In each group, four founder lines were crossed to produce six connected biparental families of segregating lines. These lines (821 and 801 for the dent and flint group, respectively), were genotyped for approximately 20k SNPs and were crossed according to an incomplete factorial design to produce 951 dent-flint hybrids, evaluated for silage performances in eight environments. Hybrid genetic variance decomposition showed a predominance of general (GCA) over specific (SCA) combining abilities. SCA explained between 13.8 and 22.6% of the within-population hybrid variance, depending on the trait. QTL detection was carried out for GCA and SCA using different models considering allelic effects transmitted from each founder lines (linkage analysis) or considering directly SNP alleles (linkage disequilibrium mapping) assuming equal or different effects in each group. In total, between 42 and 54 QTLs were detected depending on the model, among which 12 to 31% presented dominance/SCA effect significant at a 5% individual risk level. Only 16 QTL were detected by all three models illustrating their complementary. Most of the QTL (about 80%) were specific to one group, consistent with the long term divergence between the dent and the flint groups. These results open interesting prospects for revisiting the concept of reciprocal recurrent selection with genetic markers.

This study was conducted in the frame of Promaïs project SAMMCR, involving INRA, Caussade Semences, Euralis Semences, Limagrain Europe, Maïsadour Semences, Pioneer Genetics, R2n and Syngenta seeds.

**P289**

# Genome-wide association study of carotenoid and tocochromanol levels in sweet corn kernels

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Higher levels of carotenoids (provitamin A, lutein, and zeaxanthin) and tocochromanols (vitamin E and antioxidants) in the U.S. food supply would help in the prevention of health complications including macular degeneration, increased risk to cardiovascular disease, and specific cancers. Sweet corn is the 3rd most commonly consumed vegetable in the U.S., but typically does not make significant contributions to daily intakes of tocochromanols and carotenoids. For both trait sets, we are leveraging findings from diverse dent corn inbred lines—both genome-wide association studies (GWAS) and predictions of breeding values based on the major quantitative trait loci (QTL) detected in association and linkage studies—to develop nutrient-dense sweet corn varieties and thereby help enhance nutritional quality. This project has collected data and biological samples from ~400 sweet corn inbred lines, which capture the major patterns of genetic diversity found in the U.S. germplasm pool, grown in replicated field trials in NY and WI during the summers of 2014 and 2015. This diversity panel was scored in both locations for phenological, agronomic, and kernel composition (major constituents) traits. Additionally, self-pollinated ears with immature (milk stage) kernels were harvested for analysis of carotenoid and tocochromanol levels using high-pressure liquid chromatography. A GWAS is being conducted to identify causal genes and favorable alleles controlling levels of these kernel metabolites. The genomic regions showing strongest association in GWAS will in turn be targeted in genomic selection models given the relative oligogenicity of these traits. These models will be used to establish a breeding population in which model predictions will be validated, re-trained, and used in selection. We are working towards developing an improved, nutrient-dense sweet corn germplasm pool, with the ultimate goal of having a positive impact, accelerated through genomics, for sweet corn breeders, growers, and consumers throughout the U.S.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), NIFA, Hatch, Cornell University Startup Funds, CAPES Brazil

**P290**

## Genome-wide dissection of the maize ear genetic architecture using multiple populations

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Improving grain yield is an essential long-term goal of maize breeding to meet continual and increasing food demands worldwide, but the genetic basis remains unclear. We used 10 different recombination inbred line (RIL) populations genotyped with high density markers and phenotyped in multiple environments to dissect the genetic architecture of maize ear traits. Three methods were used to map the quantitative trait loci (QTLs) affecting ear traits. We found 17-34 of minor- or moderate-effect loci that influence ear traits, with little epistasis and environmental interactions, totally accounting for 55.4-82% of phenotypic variation. Four novel QTLs were validated and fine mapped by using candidate gene association analysis, expression-QTL analysis and heterogeneous inbred family validation. Combining multiple different populations is a flexible and manageable way to collaboratively integrate widely available genetic resources, thereby boosting the statistical power of QTL discovery for important traits in agricultural crops, ultimately facilitate the breeding programs.

Funding acknowledgement: National Hi-Tech Research and Development Program of China (2012AA10A307), National Natural Science Foundation of China (31525017, 31222041, 31401389), National Youth Top-notch Talent Support Program.

**P291**

## Genome-wide Study of Genes Controlling Phenotypic Plasticity of Root Architecture to Nitrogen in Maize

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Modern intensive agricultural practices exemplify the use of substantial inputs of nitrogen fertilizer to achieve high yields. But this comes at cost of significant nitrate leaching and greenhouse gas emissions from soil. To mitigate these effects, we need to develop varieties that are more nitrogen use efficient(NUE). As the primary means of nitrogen acquisition, understanding root systems will be critical to this effort. Changes in root system architecture(RSA) can affect the capacity to acquire nutrients and water. ViceVersa, nutrient availability can also play important roles in regulating RSA.
Using 'shovelomics' and image analysis, we showed nitrogen(N) availability can have an enormous impact on maize RSA. We screened a set of diverse hybrids/inbreds under two N_levels at UIUC_2014/2015. Several RSA showed particularly high variations in their morphological response to different N levels, including ProjectedRootArea, StemDiameter, RootTipNo. Besides, NSS(non-stiff stalk) and SS(stiff stalk) responded differently to different N_levels. Generally, NSS showed the ability to achieve higher biomass at low N, but is lacking in its response to fertilizer N. In contrast, SS has the ability to respond to applied N, but is not as tolerant of low N.
Additionally, using a combination of lab-based 3D root imaging and shovelomics, we identified candidate genes controlling RSA by GWAS. Our mapping population was Illinois Protein Strain Recombinant Inbreds(IPSRIs), one of the important outputs of Illinois Long Term Selection Experiment. This population demonstrated differences in their nitrogen uptake capacity. Thus, we hypothesize that future validation of these candidates will lead to an understanding of the genetic control of NUE root ideotypes in maize.

Funding acknowledgement: National Science Foundation (NSF)

**P292**

## Genotypic diversity in yield and grain quality responses to elevated ozone of diverse inbred and hybrid maize

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Oxidative stress from exposure to tropospheric ozone ($O_3$) currently causes significant yield losses in the world's major crops. This represents economic losses of ~$14 to $26 billion and is predicted to increase as pollution emissions increase in key growing regions this century. However, little is known about the genetic and genomic basis for yield loss to oxidative stress in any crop, but especially $C_4$ crops like maize. The yield and grain quality of maize were compared under ambient (~40 ppb) and elevated $O_3$ concentrations (100 ppb) over two growing seasons at the Free Air Concentration Enrichment (FACE) site in Champaign, IL. In 2014, 52 inbred lines representing the extremes of $O_3$ sensitivity were tested in addition to 26 hybrids. In 2015, 10 inbred lines were re-tested in addition to 8 hybrid lines. Ear mass was, on average, significantly lower in inbred (-7%) and hybrid (-9%) lines. But, some lines were sensitive to yield loss (up to -76% in inbreds and -26% in hybrids) while others were highly tolerant of growth at elevated $O_3$. Yield loss was primarily driven by decreased kernel number on inbreds versus decreased individual kernel mass in hybrids. Yield loss was also associated with increased kernel protein content under elevated $O_3$. Current analyses are evaluating kernel growth rate and grain-filling period to assess whether reduced photoassimilate supply or a shorter grain filling period causes yield loss.

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

**P293**

## GMATA: a powerful tool for microsatellite characterization, marker development and graphic display applied to the maize genome

(submitted by Shumeng Zhang <sz88391@uga.edu>)
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Abstract:
Simple Sequence Repeats (SSR), also called microsatellites, are highly variable tandem DNA sequences that are widely used as genetic markers. The ever-increasing number of whole genome sequences provides resources for SSR marker development. A major challenge is the lack of efficient software to discover and display SSR information on a genome scale. Our Genome-wide Microsatellite Analyzing Tool Package (GMATA) is a novel software that integrates SSR mining, statistical analysis, marker design, and marker display with other genome features, marker polymorphism screening, and investigation of marker transferability at the whole genome level. Only one input file of DNA sequence data is needed. GMATA applies novel strategies for SSR analysis and primer design in large genomes, which makes it run much faster and more accurately than existing tools. It is also capable of mining SSR in DNA sequences of any size on a standard desktop computer. GMATA, programed in Perl, Java and R, is user friendly with only clicks in the graphic interface or on the command line, and is executable in multiple computing platforms (Windows, Linux and Mac). We present examples applying GMATA to the maize genome. In summary, GMATA is the first application tool that powerfully bridges SSR at the whole genome scale for markers to geneticists and breeders.
Availability: The GMATA source code is freely available online at
http://sourceforge.net/projects/gmata/?source=navbar
Keywords: genome; SSR marker development; polymorphism; statistical plotting; marker displaying

Funding acknowledgement: National Science Foundation (NSF)

**P294**

## Haploid Inducer Development for Specialty Corn

(submitted by Thomas Lubberstedt <thomasl@iastate.edu>)
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Modern line development in maize relies heavily on in vivo maternal haploid induction, since haploid inducing lines with reasonable high induction rates became available. The improvement of haploid inducing lines both agronomical and in respect to their adaptation to different environments became necessary. As the DH technology is applied to a more and more diverse germplasm, special requirements as additional phenotypic marker for haploid selection, or inducer lines able to overcome partial sterility in for example popcorn or organic corn, emerged.
At the Doubled Haploid Facility at Iowa State University (http://www.plantbreeding.iastate.edu/DHF/DHF.htm) we developed BHI306, a specialty corn haploid inducer line able to overcome dent sterility (Ga1), and with an additional phenotypic marker Pl1 – for haploid selection based on the root color.

Funding acknowledgement: National Science Foundation (NSF)

**P295**

## High-throughput Image-based Phenotypic Analysis of Tassel Morphology in Maize

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As producers of pollen, maize tassels are one half of the reproductive system vital for inbred maintenance, hybrid creation, and agricultural grain production. Within hours of removal from the plant the tassel's natural shape begins to deform, making storage and subsequent phenotyping difficult and imprecise. Additionally, the size and shape of tassels can be difficult to quantify with traditional phenotyping tools. These characteristics make tassels an ideal candidate for image-based phenotyping. The goal of this project was to use high-throughput imaging to immortalize maize tassels for extended future study and for quantification of traits that are difficult or impossible to measure by hand. In the summer of 2015, we imaged ~7,700 tassels from 750 inbred lines belonging to the Wisconsin Diverse (WiDiv) diverse panel and 594 inbreds derived from three biparental populations sharing a common parent. These populations were selected based on observed divergence between the parents for key tassel traits. Genotypes were also evaluated for hand-measured traits (branch number (BN), tassel length (TL), and spike length (SL)) in replicated field experiments across three environments in WI during 2013, 2014 and 2015. Substantial diversity was observed for hand-measured traits. In the WiDiv, BN ranged from 0 to 48, TL from 16cm to 56cm, and SL from 6cm to 38cm. In the biparental populations, BN ranged from 1 to 51, TL from 14cm to 49cm, and SL from 11cm to 37cm. Images were analyzed by custom scripts with the intention of corroborating the hand-measured traits and defining new morphological traits that cannot be easily quantified by physical measurements, such as spike curvature and tassel compactness. These analyses are intended to form the basis for future mapping projects as well as to contribute to further study of maize reproductive diversity and its potential relationship to overall productivity.

Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE), Great Lakes Bioenergy Research Center (GLBRC)

**P296**

# High-throughput phenotyping and genotyping to dissect canopy architecture in maize

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Improved canopy architecture is one of the ways maize hybrids have adapted to higher planting densities. Modern hybrids have more upright leaves when compared to older hybrids. Canopies containing upright leaves distribute light more effectively throughout the canopy. This study is being conducted to further the understanding of maize canopy architecture, specifically looking at how differences in leaf angle (LA) affect yield and how light is distributed throughout the canopy. PHW30 was identified as having an upright LA (75.9°), whereas B73 (63.1°) and Mo17 (55.7°) have a flatter LA relative to PHW30. Four reciprocal bi-parental populations (upright x flat) were developed, and LAs for the $F_2$ progeny were measured. Subsets (flat, average, and upright) of $F_2$ lines for each population were selected and genotyped. From these selected lines, we used the double haploid process to produce inbred lines. Extensive genotyping and phenotyping with an unmanned aerial vehicle (UAV) and individual plant image analysis will be used to characterize the inbred lines into phenotypic groups and for QTL mapping. In addition, the favorable LA QTL are being backcrossed from PHW30 into B73 and Mo17. Four different hybrid combinations will be made with the modified and unmodified versions of B73 and Mo17. They will be extensively phenotyped (UAV and individual plant image analysis) and tested for yield. In a companion project, we utilized publically available genomic and phenotypic data sets to predict LA for a subset of the Ames Panel. We measured LA in that subset, which allowed us to calculate prediction accuracy for our model. Improving canopy architecture is one of many approaches necessary for yield improvements in maize. We outlined a procedure to use high throughput genotyping and phenotyping across different types of mapping populations to dissect this quantitative trait.

Funding acknowledgement: National Science Foundation (NSF)

**P297**

# Identification of Aflatoxin Resistance and High Yield Potential in Maize Hybrids in the Southeast Regional Aflatoxin Trials (SERAT)

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Aflatoxins pose a potential serious health hazard to humans and livestock, requiring significant economic cost in identifying and disposing of contaminated grain. Since 2003, a multi-environmental trial of public breeding maize (*Zea mays* L.) hybrids across multiple programs in the southeastern United States has evaluated stable resistance to the aflatoxin accumulation following inoculation with *Aspergillus flavus*. The Southeast Regional Aflatoxin Trial (SERAT) was formed to identify public breeding material with the most consistent resistance to aflatoxin accumulation, and to evaluate their essential agronomic traits in different environments. Yield, plant height, days to flowering and lodging were among the traits evaluated, and in four of the locations, levels of aflatoxin in ears that had been previously inoculated with a suspension of the *A. flavus* spores were determined. From 2006 to 2014, the average yield over all environments and genotypes was 8.4 t/ha, with the check average of 10.1 t/ha, exceeding the research program average of 8.1 t/ha by twenty percent. The program entries however, exhibited lower levels of aflatoxin on average even before log transformation at 336 ppb versus 377 ppb for the checks. Program averages are less important than finding a better performing line, and when comparing the top five program hybrids with the check average in a given year, some yielded on par with the checks and some had aflatoxin levels significantly lower than the checks. Most of the hybrids were tested as a unique set in different locations for a given year; repeatability and stability were evaluated by year as well. Repeatability for yield ranged from .70 to .86, and log transformed aflatoxin ranged from .45 to .80. Type II stability as measured by joint regression analysis revealed a consistent negative correlation between high yielders and degree of stability in all but one year (2009).

Funding acknowledgement: United States Department of Agriculture (USDA)

**P298**

## Identification of genes associated with quantitative and multiple disease resistance in maize

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Southern leaf blight (SLB, causal agent Cochliobolus heterosrophus), northern leaf blight (NLB, causal agent Exserohilum turcicum) and grey leaf spot (GLS, causal agent Cercospora zeae-maydis) are important maize foliar fungal diseases. Resistance to these diseases is largely quantitative in nature. We have used linkage and association mapping combined with utilization of the UnifomMu insertional mutation resource to identify and characterize loci and genes for resistance to SLB and to multiple diseases. A genome wide association study for SLB resistance in the NAM and other mapping populations identified about 300 candidate genes for SLB resistance. We identified insertional mutants in 128 of these genes and screened these for alterations in resistance to SLB, NLB and GLS. Lines mutant in nine of these genes had altered SLB resistance. Several of these lines were also altered in resistance to NLB and GLS. In particular, multiple lines of evidence suggest that a caffeoyl-CoA O-methytransferase (CCoAOMT) gene, *ZmCCoAOMT2*, associated with lignin biosynthesis, is important for both SLB and GLS resistance. Our group has also identified *ZmCCoAOMT2* as a gene associated with modulation of the hypersensitive defense response (HR). We observed correlations between the effects of the different NAM parent *ZmCCoAOMT2* alleles on resistance and HR, suggesting that the gene's effect on resistance may be modulated through its effect on HR. Transgenic validation of the effect of *ZmCCoAOMT2* is underway.

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

**P299**

## Identification of Genetic Background Modifiers of the Maize *brown midrib3 (bm3)* Mutant

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Developing sustainable forage and biofuel crops such as maize involves improving the productivity and biochemical composition of the biomass. Forage composition can be greatly affected by the introduction of major-effect genes, such as the *brown midrib3 (bm3)* mutant. This mutant disrupts a relevant step of the lignin biosynthetic pathway. While *bm3* genotypes have reduced recalcitrance, substantial variation on yield and overall plant morphology have been observed depending of the genetic backgrounds in which *bm3* is introduced. The objective of this research is to test the hypothesis that endogenous genetic background variants differentially affect the penetrance of this single large effect gene, which, subsequently impacts overall plant performance. To contribute to this evaluation, 135 F3:F4 families were generated from the cross of inbreds W64A*bm3* and W182E*bm3*, which demonstrated distinctive responses to the introduction of *bm3*. These F3:F4 families were crossed by the isogenic types, *bm3* and wild-type, of both parent lines. The resulting 540 hybrids generated were evaluated in a replicated field trial to assess digestibility and other relevant agronomic traits. Significant differences in digestibility, biomass yield, plant and ear heights, and flowering time were observed among genotypes. Differences between *bm3* and their wild-type counterparts were significant for digestibility, biomass, and flowering traits. *bm3* plants expressed delayed flowering time, lower biomass, and increased digestibility when compared to their wild-type counter parts. When comparing *bm3* crosses, the phenotypic responses were seen to affect W182E*bm3* hybrids more dramatically than their W64A*bm3* counterparts in terms of delayed flowering time, plant and ear heights, and biomass. Genetic mapping is being completed to identify and compare possible QTLs across the RILs and their crosses with the four testers as well as to investigate why W64A*bm3* genotypes appear to be superior in performance compared to W182E*bm3* genotypes.

Funding acknowledgement: United States Department of Agriculture (USDA) Hatch WIS01639, Forage Genetics International

**P300**

## Improving the nutritional quality of processed maize food products: A step toward the prevention of aging-related diseases in humans

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Over the last century, significant advancements have been made in both plant breeding and human medicine. Today, people are living longer and are less likely to face starvation due to crop failure. However, as the average age of the world population continues to increase, so does the incidence rate of aging-related diseases such as cancer, Alzheimer's Disease, and Parkinson's Disease. Aging-related diseases may be partially prevented by diets rich in beneficial phytochemicals, including hydroxycinnamic acids. Such phytochemicals can be found in produce and whole grains. However, the consumption of whole grains and fresh produce follows a socioeconomic trend. Individuals facing socioeconomic constraints are more likely to purchase starchy, calorie-dense foods than fresh produce or whole grain products. Individuals from socioeconomically poor areas are also more susceptible to developing aging-related diseases.
Maize grain typically used in the production of breakfast cereals and maize-based snack foods contains a high concentration of hydroxycinnamic acids. This study examines the possibility of breeding maize with the ultimate goal of improving the bioavailable hydroxycinnamic acid content in maize food products. We explored two possible strategies: (1) breeding for nutritional content that persists through processing and (2) breeding for all-natural food additives that could be extracted from the maize kernel. In the former case, we developed a small-scale laboratory process that mimics the maize cereal production pipeline. Whole kernels, cornflakes, and three intermediate products were evaluated for their insoluble-bound and soluble hydroxycinnamic acid content. Correlation coefficients among agronomic, milling, and nutritional traits of interest were calculated, and a quantitative genetic analysis was performed for all processing stages. We conclude that breeding for improved hydroxycinnamic acid content that persists throughout food product processing is much less efficient than breeding maize for use in all-natural food additive production.

Funding acknowledgement: Kellogg's Company, Dow AgroSciences

**P301**

## *In silico* mapping of yield quantitative trait loci (QTL) in maize

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*In silico* mapping can detect QTLs using existing phenotypic data from breeding programs. This approach utilizes the relationships between maize lines based on identity by descent (IBD) segments occurring from historical recombination events. We explored the use of *in silico* mapping in maize (*Zea mays* L.) using yield data from a North Carolina Design II breeding scheme using Stiff Stalk and Iodent commercial caliber material. The 110 hybrids were evaluated for grain yield at 3 plant densities in 3 locations over 3 years. Many of the Guelph inbred lines do not have fully described pedigrees, so genotyping by sequencing data was used to generate a genomic relationship matrix (G) for each heterotic group. A mixed linear model approach was used to test SNP marker alleles for associations with additive effects for grain yield in each heterotic pattern. No significant SNPs were found for Iodent inbreds. Stiff Stalk inbreds had 123 significant SNPs (FDR adjusted $q < 0.05$) with 121 SNPs at $q=0.0012$. The genetic variation is found on 6 chromosomes and is represented by 56 gene models, only eight of which have proposed functions. Six of the gene models are transcription factors, while the other two are involved in ATP synthesis/ hydrolysis and abiotic stress response through glutathione-redox balance. We conclude that GBS data together with phenotypic data can be used for *in silico* QTL mapping in the absence of pedigree information.

**P302**

## In vitro regeneration of Sorghum plants from immature embryo – A potential tool for sorghum transformation

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Sorghum is a C4 plant and belongs to grass family. It has high photosynthetic and water efficiency and attains maturity within three months. It can grow on marginal soil with little inputs in terms of water and nutrients and is the fifth most important crop in the world. In the African and Asian nations sorghum serves as an important source for food while in Americas and Australia it is more used as a fuel due to high sugar content in its juicy stems. However, this plant is recalcitrant to genetic manipulation and very little success has been obtained in dissecting the functional genomics of this plant. The first successful transgenic sorghum was reported by Casas et al. in 1993, while the first Agrobacterium mediated sorghum transformation was reported by Zhao et al. in 2000. Optimization of tissue culture media like hormones, amino acids, gelling agents along with other parameters like selection of genotype, explants, phytotoxicity of selection agent, pre-culturing and heat shocking are reported to produce positive outcomes in sorghum tissue culture. In this experiment we regenerated 22 healthy sorghum plants starting with 200 immature embryos thus having an efficiency of 11%. This is an initial report on this technique and we are still collecting data to modify it for higher efficiency. This protocol can be useful in future to generate transgenic sorghum lines.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P303**

## Incorporation of Evolutionary Constraint Improves Genomic Prediction of Hybrid Phenotypes

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Complementation of deleterious alleles has long been proposed as a major contributor to the hybrid vigor observed in offspring of inbred parents. We tested this hypothesis using evolutionary measures of sequence conservation to ask whether incorporating information about putatively deleterious alleles can inform genomic selection (GS) models and improve phenotypic prediction. We measured a number of agronomic traits in both the inbred parents and hybrids of an elite maize partial diallel population and re-sequenced the parents of the population. We identified haplotype blocks using an identity-by-descent (IBD) analysis and scored these blocks on the basis of segregating putatively deleterious variants. We implement a genomic prediction model and show that incorporating sequence conservation, especially with an incomplete dominance model, improves prediction accuracy in a five-fold cross-validation experiment for several traits *per se*, as well as heterosis for those traits. These results provide strong empirical support for incomplete dominance (or partial complementation) in explaining heterosis, and demonstrates the utility of incorporating functional annotation and its potential in phenotypic prediction and plant breeding.

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

**P304**

# Integrating traits associated to nitrogen use efficiency into the genomic prediction of tropical maize lines

(submitted by Danilo Hottis Lyra <daniloh@iastate.edu>)
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The use of modern methods including molecular information, as genomic prediction has aided, substantially, in selecting best individuals based on the genomic breeding values (GEBV). However, to improve prediction accuracy in nutritional stress, it is essential to include the information of traits related to photosynthesis and root system. Thus, we evaluated the prediction accuracy of tropical maize lines, in normal and low level of nitrogen, with the inclusion of three covariates in GBLUP model.
Sixty lines with genetic variability for nitrogen use efficient (NUE) were genotyped with 768 SNPs. On the experimental field, the lines had their grain yield (ton.ha$^{-1}$) evaluated in two nitrogen (N) levels in the soil, normal (100 kg ha$^{-1}$) and low (30 kg ha$^{-1}$). The experimental trial was carried out in Anhembi Station in Piracicaba-SP, Brazil, during the second growing season of 2015. Variance components estimation were performed by REML and the GEBV prediction for individuals was assessed by additive GBLUP model. A second GBLUP model was tested with the inclusion of photosynthetic efficiency of PSII, axial root length (cm) and axial root volume (cm$^3$) as fixed covariates (COV-GBLUP). Data were analyzed with ASReml-R. Prediction accuracy (PAmean) was evaluated using the five-fold cross-validation with 100 times repetitions.
431 SNPs were selected to compose the markers matrix. The PAmean for GBLUP in normal and low N was 0.24. For COV-GBLUP, in normal and low N was 0.18 and 0.24, respectively. Two lines (L32 and L55) outperformed in both cropping systems. The inclusion of traits associated to NUE as covariates in GBLUP model decreased the prediction accuracy in normal N and remained the same in low N. In addition, the prediction of GEBV of tropical maize lines, in N stress condition, is a valuable tool to select potential lines to be included in crosses related to NUE.

Funding acknowledgement: São Paulo Research Foundation-FAPESP (Process: 2013/24135-2; 2014/26326-2; 2015/14376-8)

**P305**

# Integrating 'omics' data to reveal genotype-phenotype associations underlying maize seed imbibitions traits

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Imbibition, defined as the rapid uptake of water by the dry seed, represents the first phase of germination and has been shown to affect emergence rate and stand establishment, all of which are important pre-requisites affecting optimum yield potential. In these experiments, we utilized a semi-automated image-based phenotyping platform across time to measure imbibition rate *K* and percent increase in seed surface area 24 hours post-imbibing (%ISSA). We demonstrated a highly significant relationship of imbibition rate *K* with both radicle emergence time ($r$ = -0.70) and total germination percentage ($r$ = 0.70), indicating that our rapid phenotyping platform exhibits potential agronomic utility. Additionally, genome-wide association mapping was performed on both of these imbibition traits utilizing ~437K RNA-seq based markers across 500 diverse maize inbred lines to identify candidate trait-associated SNPs. Using a systems biology-based multi-omics approach which leveraged several public data sets, we were able to resolve genetic regions associated with imbibition rate *K* and identified two candidate genes, one encoding a putative α-amylase protein and the other a β-1,3-glucanase protein. SNP-based pathway enrichment analysis indicated over-representation of biological processes related to carbohydrate metabolism, hydrolase activity, protein degradation, translation, membrane structure, and kinases involved in signaling cascades. These terms overlapped with both our identified candidate genes and global processes known to be differentially expressed during imbibition and early germination in other plant species. Overall, this study has helped characterize the genetic architecture of seed imbibition in maize and has demonstrated the power of integrating omics datasets to better identify candidate genes underlying genotype-phenotype relationships.

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

**P306**

## Investigating the genetic basis of parallel response to selection for early flowering time in the TropicS.

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Genetic diversity is a key component to the health and success of many major agricultural crops by contributing to their ability to adapt to changing environments. Understanding the genomic response to selection is important for adapting and utilizing more diverse sources of germplasm. This study aims to gain a further understanding of the response to selection for flowering time across different environments . We conducted a multi-environment parallel selection experiment using a TROPICal Synthetic (TropicS) population developed from seven inbred lines. For two generations, standardized phenotypic selection for early flowering time was applied at each of eight locations in the U.S. (spanning from WI to PR). During selection, we sampled 1000 random plants, the 500 earliest plants (selection units), and the 500 latest plants resulting in ~32,000 tissue samples. We developed a standardized and empirically-optimized genotyping-by-sequencing protocol to score these heterozygous samples. In addition, following selection, a GxE experiment was performed to evaluate all generations from all locations at all of the original locations of selection. Together, the study design allows results from extreme QTL mapping, GWAS, selection mapping, and phenotypic responses to be integrated for insights into selection and adaptation. We will present the latest progress of this study including results for extreme QTL mapping and selection mapping, the use of read-derived haplotypes from GBS data, and phenotypic analysis of the GxE experiment.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P307**

## Investigating the unstability of yield-related QTL in maize: toward identification of genomic regions associated with tolerance to precise scenarios of heat or drought

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Crops are subjected to frequent episodes of drought and heat so grain yield of specific genotypes are largely unpredictable (GxE interaction).
We have performed a genome-wide analysis of plant performance in European fields, with 29 combinations site x year x watering regime, classified into clusters of climatic scenarios based on the time courses of temperature, evaporative demand, light and soil water potential precisely measured in each field. A panel of 244 maize hybrids, genotyped with 354k polymorphic SNPs, was analyzed in this network of experiments.
All identified Quantitative trait loci (QTLs) of yield or of its components displayed a high QTL x Environment interaction. QTLs associated with higher yield were detected in scenarios with hot temperature but not in cooler scenarios, or vice versa. Allelic effects associated with these QTLs were significantly related to temperature or to evaporative demand in the whole dataset. The same approach was performed for dry scenarios, resulting in yield-related QTLs whose allelic effects were related to soil water potential across situations. Hence all QTL displayed scenario-dependent allelic effects on yield.
Because the frequencies of each scenario used in this study were previously quantified over the European maize cropping area, the comparative advantage of each QTL can be assessed in each European region. This provides breeders with calculated probabilities that a given allele has positive, null or negative effects in different geographic regions and therefore assists design of ideotype for drought-prone environments.

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**P308**

## Maize germplasm organization and visualization according to genomewide marker effects

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Single nucleotide polymorphism (SNP) markers are frequently used to estimate genetic dissimilarity among maize lines and to predict, via genomic selection, which individuals have the highest trait values. Genetic diversity can be characterized and visualized not only at the whole-line level, but also in terms of predicted marker effects in different regions across the genome. Such visualizations can elucidate the arrangements of marker haplotypes and of chromosome segments or whole chromosomes that are enriched for favorable alleles within subsets of lines. With the increased use of doubled haploids in maize breeding, such regions or segments have a high chance of being transmitted as a unit. Beneficial haplotypes may even be located in lines that perform poorly for the trait. Thus, the ability to visualize marker effect haplotypes throughout the available germplasm can help identify potential targets for selection in the creation of doubled haploids. We applied this approach of germplasm organization and visualization to 271 historical maize lines that were genotyped with 28,626 SNP loci and phenotyped for phenological, morphological, kernel composition, and disease resistance traits. Results demonstrate that marker effect-based similarity differs from traditional SNP similarity. The approach can provide a new way to characterize germplasm, as well as visualize and identify haplotype blocks within the lines.

**P309**

## Mapping and identifying candidate genes of the modifier of amylose extender 1 (MAE1) mutation in maize (Zea mays L.)

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In maize (*Zea mays L.*), amylose extender 1 (*ae1*) mutants are associated with an increase in amylose-like starch from 25 to 70%, affecting the important quality factor of kernel hardness and levels of resistant starch. A novel maize phenotype of completely shrunken, collapsed kernels was observed when backcrossing *ae1* alleles into the food-grade inbred line cgx333. This novel mutation is recessive, epistatic to *ae1* and appears to be due to the segregation of a single gene, *modifier of amylose extender 1* (*mae1*). We hypothesize that the *mae1* gene represents a yet unknown step in starch synthesis and that in the presence of functional SBEIIb results in more amylose-like starch. Using a RIL population, the *mae1* mutation will be mapped by genotype-by-sequencing (GBS) and starch will be characterized. Strategies will be developed to test putative candidate genes inferred by the maize B73 reference genome. This research will provide novel insight into maize starch synthesis.

**P310**

## Mapping of quantitative disease resistance loci derived from recurrent selection for northern leaf blight resistance

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Considerable effort has been made to map quantitative trait loci (QTL) affecting resistance to northern leaf blight (NLB), a globally important disease of maize. These efforts may or may not have captured most of the QTL present in maize breeding germplasm. To test the extent to which these studies have captured resistance QTL, we mapped QTL derived from diverse sources through recurrent selection. A CIMMYT recurrent selection (RS) program, beginning with 411 diverse full-sib families sourced from 4 continents, yielded large gains in NLB resistance (Ceballos et al., 1991). One highly heterozygous individual from the improved population was crossed to B73 to make two unique F1's, expected to share half of their RS-derived alleles. These F1's were selfed to make two overlapping populations of recombinant inbred lines (RILs), which were genotyped by GBS and screened for NLB resistance. The two RIL populations were confirmed to share 51% of their RS-derived alleles. Selection mapping in the original RS population suggested that gains in NLB resistance were mostly due to rare alleles increasing to moderate frequency, rather than fixation of alleles. If resistance quantitative trait loci (QTL) in these two families are mostly found in regions where the two families inherited different RS-derived alleles, it would support this conclusion. Because of the diverse founding materials used, resistance alleles may be sourced from many different progenitors. GBS allows us to match resistance QTL to known maize haplotypes, as well as known resistance QTL derived from lines with these haplotypes. We will report on the extent to which QTL for NLB resistance were fixed within the RS individual and the extent to which novel resistance QTL could be detected.

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

**P311**

## Mapping variation linked to phosphate efficiency among maize landraces originating from the Purhepecha plateau of Michoacan

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Phosphorus (P) deficiency is a major limiting factor on crop production worldwide. Current practice requires the addition of large quantities of P fertilizer to maintain high yields and will not be sustainable as readily available sources of high-grade phosphate rocks become scarce. To reduce this dependency on P fertilizer and sustain future crop production, it is important to enhance P efficiency through improved P acquisition and utilization.

This project aims to evaluate the potential of maize landraces originating from Mexico's P-limiting highland volcanic soils as a source for improved phosphate efficiency. The overarching hypothesis is that there is valuable genetic diversity within these landraces that can be used to improve P efficiency in modern cultivars. To test this hypothesis, we will genetically map P efficiency in the reference Mexican highland landrace Palomero Toluqueño (PT) using a population of 100 B73 x PT $BC_1S_5$ recombinant inbred lines (RILs). In addition, we will evaluate introgression stocks derived from landrace accessions collected from the Purhepecha Plateau of Michoacan, material characterized previously to show high P efficiency. We will evaluate material in a low P field site, assessing phenology, morphology, senescence, root system architecture and elemental profiles, in addition to overall yield.

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

**P312**

## Measuring the growth and development of Maize inbreds B73 and Mo17 in a newly established outdoor corn plot after cold stress delivered in early seedling development

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Over the years, farmers have begun planting their corn crop earlier and earlier. This is due to a few different factors including the need to plant more acres and therefore get an earlier start, avoiding the heat and water stress that occurs late in the summer, and allowing the plants to grow tall enough that the ear leaf will be protected from soil and residue-borne diseases. However, earlier planting comes with the risk of the corn plants being exposed to cold temperatures, especially during the earliest stages of development. Intermated B73 and Mo17 lines can be used to identify genetic loci conferring responses to cold stress, but in order to effectively do this, a cold condition that elicits the biggest difference in response between B73 and Mo17 should be identified. The goal of this study was to determine which cold stress condition delivered within the first week of seedling development would cause the biggest difference in adult growth between B73 and Mo17. An outdoor field site was established so that the corn could be grown to maturity in a realistic environment. The site was marked, sprayed with Roundup, tilled, soil sampled, and fertilized in preparation for planting. Cold stress at 10°C or 4°C was delivered at either imbibition, germination, one day after germination, or three days after germination. Seedlings were grown on agar medium for three days. They were then transplanted to soil, hardened in a greenhouse, and transplanted to the outdoor field. Height measurements were taken until the plants tasseled and after the plants reached maturity, whole plants were removed for later dry mass measurement and the roots were imaged and analyzed using the DIRT computational pipeline. Cold stress delivered early in development was found to negatively impact growth observed in adult development.

Funding acknowledgement: National Science Foundation (NSF)

**P313**

## Metabolic Profiling of Plant Extracts Using Direct Injection Electrospray Ionization Mass Spectrometry Allows for High-Throughput Phenotypic Characterization According to Genetic and Environmental Effects

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In comparison to the exponential increase of genotyping methods, phenotyping strategies are lagging behind in agricultural sciences. Genetic improvement depends upon the abundance of quantitative phenotypic data and the statistical partitioning of variance into environmental, genetic, and random effects. A metabolic phenotyping strategy was adapted to increase sample throughput while saving reagents, reducing cost, and simplifying data analysis. The chemical profiles of stem extracts from maize plants grown under low nitrogen (LN) or control trial (CT) were analyzed using optimized protocols for direct-injection electrospray ionization mass spectrometry (DIESI−MS). Specific ions significantly decreased or increased because of environmental (LN versus CT) or genotypic effects. Biochemical profiling with DIESI−MS had a superior cost−benefit compared to other standard analytical technologies (e.g., ultraviolet, near-infrared reflectance spectroscopy, highperformance liquid chromatography, and gas chromatography with flame ionization detection) routinely used for plant breeding. The method can be successfully applied in maize, strawberry, coffee, and other crop species.

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**P314**

## Metabolite-QTL analysis of cuticular surface lipid production on maize silks

(submitted by Tes Posekany <posekany@iastate.edu>)
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Upon emergence from the husk leaves, maize silks are exposed to a myriad of environmental stresses (e.g., UV radiation, insect damage, microbial pathogenesis, desiccation). Like other aerial plant surfaces, the maize silk has a cuticle infused with and coated by surface lipids (SLs) that act as an environmental barrier. The SL metabolome includes >100 metabolites that are predominantly unbranched, non-cyclic hydrocarbons, fatty acids, and aldehydes ranging in chain lengths from 16 to 35 carbon atoms. To understand how the silk cuticle fulfills its diverse functions, we have implemented a systems biology approach to comprehensively determine the enzymes, regulators and metabolic reactions involved in silk SL biosynthesis. As an initial step, we performed QTL mapping using 254 Intermated B73xMo17 recombinant inbred lines, which possess considerable SL metabolome variation. Silk samples were collected three days after emergence from ~1,000 field grown plants (254 isolines, 4 samples/line), using only the plants that represented the mid-silk cohort in each line. SLs were extracted and quantified by GC-MS. QTL analysis of 116 constituent traits, 50 metabolite-class traits, and 35 relative composition traits identified a total of 420 metabolite-QTLs (mQTLs) that modulate the abundance and composition of the silk SL metabolome (experiment-wise threshold: $\alpha$=0.05). Inclusion of constituent traits that are precursor (fatty acid), proposed intermediate (aldehyde) and end-product (hydrocarbon) metabolites allows for a more complete characterization of the genetic network. Using confidence intervals, we demonstrate that positional resolution is very high in this experiment, with some mQTL regions harboring only tens of genes. Co-localizations of mQTLs to narrowly defined positions also suggest pleiotropic actions by the causal polymorphisms at >20 genomic locations. We will discuss how we use these results to identify and functionally characterize the contributions of the enzymes, regulators and metabolic reactions involved in SL biosynthesis.

Funding acknowledgement: National Science Foundation Award Number 1354799, USDA-ARS

**P315**

## Mild Inbreeding Depression in the Zea Synthetic Population

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Comparing inbred and outbred families is a classical way to identify deleterious alleles and genomic regions underlying inbreeding depression. In order to identify deleterious alleles from teosinte (*Zea mays* ssp. *parviglumis*) and inbred maize in a common background, we have created a set of 924 paired inbred (S1) and outbred (S0) families derived from the Zea Synthetic (ZeaSyn) population. The ZeaSyn population was created via several generations of random mating of the NAM founders and 11 geographically-distinct teosinte individuals teosinte, and should be approximately 38% B73, ~2% of each NAM parent plus Mo17, and ~1% of each teosinte. The 924 paired S0/S1 families were grown over two years at three different locations and were phenotyped for several agronomic and fitness traits. The parents of the S0/S1 families were genotyped using genotyping-by-sequencing (GBS). These genotypes were then used in several genome-wide association analyses, which uncovered significant loci for S1 families, S0 families, and inbreeding depression itself (S0 trait value – S1 trait value; Delta). We will continue to refine these analyses and also identify the donor haplotypes of the loci of interest. We expect to find a larger number of deleterious loci to originate from teosinte than from maize.

Funding acknowledgement: National Science Foundation (NSF)

**P316**

## Mining allies in exotic germplasm to modify carotenoid profile and content in tropical maize

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Maize is an ideal vehicle for provitamin-A improvement and delivery in Africa because of its broad germplasm base, widespread use in preparation of numerous traditional foods, adaptation to diverse production environments, and the potential of the crop to generate and continually provide productive cultivars that are attractive to farmers. Although yellow maize contains provitamin-A carotenoids that can be converted into vitamin A in the human body, they represent less than 25% of the total carotenoids in most commonly grown and consumed maize cultivars in Africa. Consequently, 12 exotic donor lines of high ß-carotene were introduced to develop backcrosses with elite tropical yellow endosperm maize inbred lines as recurrent parents. These backcrosses have been sources of advanced lines developed through repeated inbreeding with visual selection for bright yellow to orange kernel color with semi-flint to flint kernel texture and desirable agronomic and adaptive traits. Many of the lines originating from these backcrosses contain ß-carotene concentrations varying form 5.0 to 20.0 µg g-1 and pro-vitamin A content varying from 8.0 to 22.3 µg g-1 in their kernels. Also, these inbred lines displayed distinct carotenoid profiles. The best backcross-derived lines accumulate 23 to 313% more β-carotene and 32 to 190% more provitamin-A than their recurrent parents. Several backcross-derived lines carry the favorable alleles of the most significant functional markers of crtRB1-3T' and crtRB1-5'TE, whereas their recurrent parents were devoid of these alleles. These results demonstrate that the infusion of alleles from diverse exotic lines can have major effects on carotenoid biosynthesis in the endosperm of tropical maize inbred lines. The backcross-derived yellow to orange endosperm maize inbred lines with desirable agronomic features have been used to develop provitamin A rich hybrids and synthetics without compromising grain yield and other adaptive traits that are required to profitably cultivate maize by farmers in tropical Africa.

Funding acknowledgement: HarvestPlus Challenge Program

**P317**

## Molecular characterization of CIMMYT maize inbred lines with genotyping-by-sequencing SNPs

(submitted by Xuecai Zhang <xc.zhang@cgiar.org>)
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CIMMYT maize inbred lines (CMLs) have been widely used all over the world and have contributed greatly to both tropical and temperate maize improvement. Genetic diversity and population structure of the current CML collection and of six temperate inbred lines were assessed and relationships among all lines were determined with genotyping-by-sequencing SNPs. Results indicated that: (1) great genetic distance and low kinship coefficients among most pairs of lines reflected the uniqueness of most lines in the current CML collection; (2) the population structure and genetic divergence between the temperate subgroup and tropical subgroups were clear; three major environmental adaptation groups (Lowland Tropical, Subtropical/Mid-altitude and Highland Tropical subgroups) were clearly present in the current CML collection; (3) the genetic diversity of the three tropical subgroups was similar and greater than that of the temperate subgroup; the average genetic distance between the temperate subgroup and tropical subgroups was greater than among tropical subgroups; and (4) heterotic patterns in each environmental adaptation group estimated using GBS SNPs were only partially consistent with patterns estimated based on combining ability tests and pedigree information. Combining current heterotic information based on combining ability tests and the genetic relationships inferred from molecular marker analyses may be the best strategy to define heterotic groups for future tropical maize improvement. Information resulting from this research will help breeders to better understand how to utilize all the CMLs to select parental lines, replace testers, assign heterotic groups and create a core set of germplasm.

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**P318**

## Morphological observation of twin embryos generated by in vivo haploid induction in maize

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Doubled haploid technology has been widely used in scientific research and breeding practice in maize. However, the biology process of haploid induction is not fully understood. A special phenomenon, polyembryo, was found during maternal haploidy production. Polyembryonic kernels can be generated in maternal haploid induction in maize, including twins and triplets, of which 98% were twins. The average frequency of twins is 0.08% among 20 inbreds studied in our reaserch, none were found in control. Frequency of twins can be improved obviously by pollinating with haploid inducer, showing positive correlation with haploid induction rate, and varied by maternal materials. Systematical classification was applied to discriminate 237 twins selected in our study. All twins were divided into V-shaped（47.7%）、Y-shaped（39.2%）and ll-shaped（3%）by embryo morphology, 24 were not sure. 207 purple-purple and 30 colorless-colorless were identifed by the color of embryo, no purple-colorless were observed. We have characterized 142 twin-seedlings, including their germination and growth status. Only 4.22% of twin-seedlings showed significantly different growth status. The majority of these aberrant seedlings seemed to be aneuploids according to FCM (flow cytometry). All these results indicated that aneuploid may affect twin-seedling development. SSR(Simple sequence repeat) and SNP(Single nucleotide polymorphisms) were used to analyze twin-seedling genotype. 50 various types of twin-seedlings showed the same band type with 30 SSR markers.13 pairs of 15 different types of twin-seedlings had the identity rate over 90% with 3K SNP markers. Identical genotype inferred that twins may be divided from zygote. In all, polyembryo arosed during haploid production, this study is helpful for better understanding the biology process of maternal haploid induction.

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**P319**

## Multivariate Prediction and Optimization of Validation Population in Genomic Selection

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Genomic selection is widely adopted in the seed industry. It usually generates predictions for a large number of untested accessions for multiple traits. Therefore, how to select promising materials from many candidates to be further tested and how to benefit from the multiple traits are interesting questions to breeders. Here, we investigated these questions in two plant populations: the maize Ames panel (a set of 3,056 diverse inbred lines), and the biomass sorghum panel (a set of 962 diverse photoperiod sensitive accessions). High-density molecular markers (435,713 SNPs for maize and 340,496 SNPs for sorghum) and multiple traits (three shoot apical meristem related traits for maize and eight biomass yield related traits for sorghum) are available to these panels. Multivariate predictions were conducted to assess the potential improvement in prediction accuracy by fitting multiple traits to the prediction model simultaneously. In addition, three optimization parameters for validation population were evaluated: prediction error variance (PEV), reliability, and upper limit of predictive ability (U). With a good understanding of these questions, we can further focus on optimization of testing site and phenotyping platform to tackle questions such as prediction in the context of broad germplasm and genotype by environment interaction.

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

**P320**

## Parental analysis of sibling genomes from a maize breeding program

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In conventional maize breeding programs, the first step in developing new inbred lines is to create a breeding cross by intermating two (or more) parental inbred lines. The breeding cross is then inbred either through a double-haploid process or through the traditional approach involving rounds of self-pollination and concurrent selection. Occasionally these breeding crosses may involve parental lines from more than one heterotic pattern (e.g., Stiff Stalk, Iodent, Lancaster). Using Genotyping-by-Sequencing (GBS) data and pedigrees it is possible to now gain insight into how the inbreeding and selection process impacts parental linkage blocks. How different are the genomes of lines derived from the same parents? In an interheterotic pattern breeding cross, how do the parental genomes assort to give rise to inbred lines belonging to one of the heterotic patterns? This study utilized 8 siblings derived from an interheterotic pattern breeding cross and 3 siblings derived from a 3-way cross hybrid to examine these questions. The inbred lines and their parents were assessed at 955,690 loci using GBS. Between 16,978 to 71,341 SNPs with an unambiguous parent of origin were identified for each individual. Among the inbred lines derived from the 3-way cross, the number of SNPs from each parent is significantly different than expectations (i.e., 2:1:1). Ideograms showing parental SNPs along the chromosomes were created for each inbred line using PhenoGram. Our results show that full siblings can vary greatly in the pattern of parental SNPs over their genome. That linkage blocks can be smaller than expected, indicating higher rates of recombination than expected, possibly driven by selection pressure. And finally that inbred lines belonging to 1 heterotic pattern can contain extensive linkage blocks from other heterotic patterns. This research demonstrates the impact of selection in shaping the pattern of parental blocks across the genomes of inbred lines derived from interheterotic pattern and 3-way breeding crosses.

**P321**

## Phenotypic variation and genetic dissection of silage yield and compositional traits in recombinant inbred testcrosses in maize. (*Zea mays* L.)

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Maize silage provides many farmers producing or raising livestock with a high energy forage. This is particularly important in states like Wisconsin where close to 20% of the total maize acreage harvested is dedicated to silage production (USDA, 2015). The goal of this study was to conduct a genetic dissection of forage yield and key compositional traits for 516 maize recombinant inbred lines (RILs) derived from three different populations [149 intermated B73 x Mo17 (IBM), 183 Oh43 x W64a (OWRI), and 184 Ny281 x H99 (NyH)] evaluated as testcrosses using inbred PHG47 as the tester. The RIL testcrosses were evaluated in a replicated field trial across three environments in 2012 and 2013 in South Central Wisconsin. Forage dry matter yield ranged from 2.064 to 11.5 ton/acre for IBM, 3.173 to 10.53 ton/acre for OWRI, 3.078 to 10.85 ton/acre for NyH and percentage of dry matter biomass ranged from 25.82 to 61.35% for IBM, 31.73 to 56.77% for OWRI, and 32.92 to 59.32% for NyH. Among the most relevant compositional characteristics, percentage of crude protein ranged from 6.84 to 11.25% for IBM, 6.37 to 10.55% for OWRI, and 6.42 to 10.5% for NyH. Mean values of those traits were relatively similar between populations. A quantitative trait loci (QTL) for percentage dry matter biomass was identified in chromosome 1 for IBM, chromosomes 1 and 3 for OWRI, and chromosome 7 for NyH. The identification of genomic regions associated with these traits could help in the development of superior maize silage varieties.

Source: USDA. Crop production 2014 summary. 2015.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P322**

## Production of Chromosome Segment Substitution Lines for the identification of multiple disease resistance loci in Maize

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Southern Leaf Blight (SLB), Northern Leaf Blight (NLB), and Gray Leaf Spot (GLS) caused by Cochliobolus heterostrophus, Exserohilum turcicum, and Cercospora zeae-maydis respectively, are among the most important corn diseases worldwide. We have previously demonstrated strong genetic correlations between resistance levels to each of these diseases in a population of 282 diverse maize inbred lines. The goal of this study is to identify loci underlying elite levels of resistance observed in 4 of the multiply disease resistant (MDR) lines identified previously by the creation of chromosome segment substitution line (CSSL) populations in which a whole genome tiling path of introgressions from MDR lines is captured in multiple disease susceptible (MDS) genomic backgrounds. Four MDR lines (NC304, NC344, Ki3 and NC262) are being used as donor parents and two MDS lines (Oh7B, H100) as recurrent parents, to produce eight CSSL populations comprising 1750 inbred lines in total. Every population was assessed for every disease in replicated trials in two environments. Phenotypic and QTL analyses were carried out using R and IciMapping softwares respectively. Moderate to high heritabilities were observed (0.32 to 0.83). Several lines in each population were significantly more resistant than the susceptible parental lines for each disease. For most populations and most disease combinations, significant correlations were observed between disease scores and marker effects and the number of lines that were resistant to more than one disease was significantly higher than would be expected by chance. QTLs for disease resistance were detected; 37 for SLB, 17 for NLB, and 21 for GLS. Among these, 30 QTLs were associated with variation in resistance to a single disease, 18 to two disease (SLB/NLB: 6, SLB/GLS: 7, NLB/GLS: 5), and 3 QTLs were associated with resistance to all three diseases.

Funding acknowledgement: United States Department of Agriculture (USDA), Fulbright, NCSU

**P323**

## QTL by Environment Interactions Underlying the Kernel Ionome in Maize

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Plant elemental profiles are determined by interactions between a plant's genetic information and its growth environment. In order to investigate genotype by environment interactions, we analyzed the maize (*Zea mays* L.) kernel ionomes of intermated B73 x Mo17 (IBM) recombinant inbreds grown in ten different environments spanning a total of six locations over five years. We measured the levels of 20 mineral nutrients in seeds from these growouts using inductively coupled plasma mass spectrometry (ICP-MS). Quantitative trait locus (QTL) mapping with element composition data and a set of 4,217 biallelic markers was implemented in the R package R/QTL. In order to consider the ionome as a network, principal components analyses (PCA) were performed with element profile data in each environment separately. QTL mapping on principal components, a complementary method to mapping on element profile data, identified additional loci affecting the ionome that were not detected in single element analysis, suggesting pleiotropic alleles with multi-element effects. We identified QTL by environment interactions (QEIs) through three methods: linear modeling with environmental covariates, QTL analysis on trait differences between growouts, and QTL analysis on factors derived from a heuristic model of ionome variation across environments. Overall, we were able to identify 147 QTL and several instances of QEI, indicating that elemental profiles are highly heritable and responsive to the environment. Weather data and other location-specific variables were analyzed to identify potential environmental drivers of ionomic variation.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P324**

## QTL Mapping for levels of β-cryptoxanthin in a Biparental Population

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Carotenoids are a family of important compounds that are produced by organisms such as bacteria, fungi and plants, but not humans. Their role in plants is to provide a source of antioxidants, hormone precursors and essential components in photosynthesis. In humans, carotenoids serve to reduce the likelihood of chronic diseases like cardiovascular disease, certain cancers, cataracts, xerophthalmia and most importantly Vitamin A deficiency (VAD). For these reasons, biofortification breeding programs seek to enhance staple crops with Provitamin A (proVA) carotenoids β-carotene, β-cryptoxanthin and α-carotene. β-carotene has been the target of many studies, and it is considered the main source of vitamin A, Yet there has been new interest in breeding for β-cryptoxanthin, as although it only has one Provitamin A structure versus two for beta-carotene, it may be twice as bioavailable as β-carotene due to the hydroxyl group. Thus β-cryptoxanthin may be as effective as beta-carotene in providing Vitamin A. The aim of this research was to better understand the genetic variation of β-cryptoxanthin and other carotenoids and identify new candidate genes using quantitative trait locus (QTL) mapping in a biparental population derived from inbreds Hi27 and A272 that both have high levels of β-cryptoxanthin. Composite interval mapping was completed to detect QTL. Four QTL were identified on chromosome 2, 5, 7 and 9. QTL on chromosome 7 and 9 have underlying candidate enzymes in the carotenoid biosynthetic pathway that have been reported. Notably QTL on chromosome 2 and 5 have not been reported. Further details and possible candidate genes will be presented. This new information can be used to enhance biofortification breeding programs for increasing proVA carotenoids in maize throughout the world.

Funding acknowledgement: National Science Foundation (NSF), HarvestPlus, CONACyT

**P325**

## QTL mapping of branching traits in Setaria populations grown in multiple environments, and comparison of QTL to those found in switchgrass, sorghum and maize

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Setaria is a C4 panicoid grass, related to switchgrass, pearl millet, sorghum and maize. It has gained support in recent years as a model for C4 photosynthesis and architectural and flowering traits, primarily because mutant and candidate gene analysis is facilitated by the small size, transformability, rapid life cycle, and availability of genome, transcriptome, and GWAS resources for the wild species *S. viridis* (green foxtail). We have been examining whether biparental crosses between *S. viridis* and the domesticated *S. italica* (foxtail millet) are useful for identifying QTL and candidate genes of interest for agronomically important traits. We are particularly interested in the environmentally labile trait of plant branching, and have been using a RIL population derived from a cross between the two species to identify branching QTL from trials conducted under controlled environment conditions (where the small size of Setaria plants are a great advantage) and from more agronomically representative field conditions. We present QTL mapping data from individual and combined trial data and show that many of the QTL are conserved between environments, suggesting that Setaria populations grown in controlled conditions can provide useful information about phenotypic response in more variable environments. In addition, comparative genomic analysis of QTLs in Setaria with those in switchgrass, sorghum and maize reveal that Setaria QTL identified in multiple environments are more likely than QTL from individual trials to be syntenous with those for branching in other species.

Funding acknowledgement: National Science Foundation (NSF), Oklahoma Center for the Advancement of Science and Technology (OCAST)

**P326**

## Quantitative genetic analysis of the NC350 x B73 recombinant inbred line (RIL) population using single-kernel near infrared spectroscopy (NIR)

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Seed composition traits are important to nutritional quality, disease resistance, and overall yield of grain crops. We analyzed genetic factors that contribute to kernel composition traits in tropical and temperate maize in a quantitative trait locus (QTL) study of the NC350 x B73 RILs. These RILs are part of the Nested Association Mapping (NAM) population, and prior joint-linkage analysis identified several QTL affecting oil, protein, and starch composition. Kernel composition traits were predicted with near-infrared spectroscopy on single kernels in maize. Single-kernel NIR was also able to predict kernel density, shape traits such as volume as well as individual kernel weight. QTL were located by using R/qtl with a 1,106 SNP marker genetic map. Some of the QTL we identified in this study overlap with genes and QTL that are already known to affect kernel composition in maize. However, we also identified novel genomic regions that specifically contributed to trait variation in the NC350 RIL population. The known and novel QTL were collectively found to explain as much as forty percent of individual trait variation. Epistatic interactions between QTL were observed for multiple traits, including protein composition and kernel density. New QTL were identified beyond those previously reported from joint-linkage mapping of the NAM population. These novel QTL may be environment-dependent or may reveal different sensitivity and specificity of quantitative trait mapping using individual RIL populations with genetic maps specific for the population. Higher resolution QTL mapping will be implemented with genotyping-by-sequencing available from the Maize Diversity Project.

Funding acknowledgement: National Science Foundation (NSF)

**P327**

## Quantitative trait loci (QTL) for reducing aflatoxin accumulation in corn

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Aflatoxin produced by Aspergillus flavus in corn poses significant health risks for both humans and livestock. Corn growers suffer huge economic losses due to increased aflatoxin accumulation especially under drought and higher temperature stress conditions. Development of resistant corn inbreds and hybrids is a sustainable way to reduce aflatoxin contamination. Identification of quantitative trait loci (QTL) for reducing aflatoxin in kernels from resistant germplasm can accelerate development of aflatoxin resistant corn using marker-assisted selection. An F2:3 mapping population developed from the cross involving a resistant inbred Mp715 and B73, a susceptible and widely used inbred for hybrid development, was evaluated in replicated field trials with artificial inoculation for two years to identify QTL for reduced aflatoxin accumulation. Using composite interval mapping, 4 to 8 QTL for aflatoxin content were identified in both years with contribution of individual QTL ranging from < 1 to 10% of phenotypic variation. More QTL were detected for husk cover with phenotypic variance range of <1 to 16% explained by individual QTL. Both B73 and Mp715 alleles at these QTL loci for both traits contributed to resistance. The husk cover and aflatoxin were significantly correlated in both years and this observation was further supported by overlapping of QTL in three genomic regions on chromosomes 4, 8 and 10, where aflatoxin resistance QTL were reported in previous studies. Since most of the QTL were of low to moderate effects, pyramiding of these QTL will be required to improve resistance to aflatoxin accumulation.

**P328**

## Quantitative Trait Locus (QTL) Mapping of Domestication Traits in Multiple Maize-Teosinte $BC_1S_4$ Populations

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Maize (*Zea mays* ssp. *mays*) was domesticated from teosinte (*Zea mays* ssp. *parviglumis*) in the Balsas river valley at about 9,000 years ago. Multiple traits were selected during the process of domestication, resulting in tremendous differences between maize and teosinte in terms of plant and ear architectures. The genetic architecture of many of these domestication traits have been previously characterized using quantitative trait locus (QTL) mapping. Several important genes regulating domestication traits have also been identified, such as *teosinte branched 1* (*tb1*), *grassy tillers 1* (*gt1*), and *teosinte glume architecture 1* (*tga1*). Different mapping populations often carry different sets of QTL, and thus, results from each mapping experiment may not overlap completely. We constructed 3 maize-teosinte hybrid ($BC_1S_4$) populations using 3 different teosinte inbred lines (TIL01, TIL03, TIL11) backcrossed to maize W22. We scored multiple plant and ear architectural traits in these populations and performed QTL mapping on each population separately using R/qtl. Here, we report our most recent findings on the QTL mapping of these $BC_1S_4$ populations.

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

**P329**

## Quantitative Trait Polymorphisms Emerging from Doubled Haploids Maize Lines.

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Doubled haploids are useful in plant breeding because they are expected to be completely homozygous. Except for rare mutations, their progeny should show no genetic diversity. Even so, over 50 years ago George Sprague and his associates demonstrated that heritable variation in quantitative traits quickly emerged among the progeny of doubled-haploids. The rate of variation was demonstrated to be greater than the rate of spontaneous mutations. The means to determine the cause of that variation was not possible at that time, but we believe it is today. We have established ten separate lineages that have descended from a single doubled-haploid B73 plant. In the summer of 2014 we planted seed for two sequential self-fertilized generations from each of these lineages. These were planted in triplicate in a randomized complete block design (RCBD). The resulting plants were evaluated for 14 quantitative traits (plant height, number of tassel branches, 100 grains weight, etc.). A heritable polymorphism for any particular trait is indicated if there is no significant difference between the two generations of a lineage but the lineage is significantly different from other lineages. An analysis of variance (ANOVA) indicates that out of 14 quantitative traits, eight are found to be significantly different; heritable polymorphisms were demonstrated by two traits: number of tassel branches and total number of kernel per year. To further test the heritability of these quantitative traits, seeds obtained from self-fertilized plants that were found to be polymorphic in 2014 were sown in summer 2015. Traits are being analyzed and compared with the 2014 observations. Polymorphisms were also seen among different generations of same lineage. This instability of phenotype may implicate epigenetics as a potential source variation. Confirmation of Sprague's results will encourage us to pursue molecular analysis.

**P330**

## Root Architecture of maize (Zea mays) in conditions in different concentrations of phosphorus.

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The root architecture affects the efficiency of the plant to take nutrients from the soil and it has a great plasticity to adapt to different kinds of environments. Typical changes in root system architecture due to phosphorus deficiency include superficial angle and branching of the crown roots, more density of root hairs, great dispersion and a greater number of the lateral roots. QTL analysis has identified different regions that are correlated with efficient root traits in low phosphorus. To discover new QTL in response to low phosphorus we used the NAM lines CML228 (efficient in low phosphorus), B73 (inefficient in low phosphorus) and 160 RILs of CML228 X B73. The plants were planted in three different levels of phosphorus 1uM, 100uM, 1000uM and harvested 35 days after germination. Roots were photographed, measured manually to obtain number of crown roots, number of whorls and weighed each fifteen centimeters of the root structure and the photos analyzed to obtain root traits like density, area of the root structure. We will present advances in the evaluation of phenotype; CML228 has higher production of root in superficial levels and more superficial angle than B73 in low phosphorus, also some RILs shows better response than the parents with more production of biomass in root, crown angle more superficial and higher production of crown roots.

Funding acknowledgement: CINVESTAV-Irapuato,CONACYT

**P331**

## Root morphology and phosphate homeostasis in maize: the role of the *Zm**Med12a*** and *Zm**Med12b*** genes

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Mediator is a muiltiprotein complex highly conserved in all eukaryontes that functions as either a transcriptional activator or repressor in developmental and physiological process, include the response to abiotic and biotic stress. It is organized into head, middle and tail modules, as well as the additional detachable CDK8 kinase module that consists of MED12, MED13, CDK8 and CYC C. Recently, in our lab, it was found that loss of MED12 in Arabidopsis results in derepression of miRNAs that respond to phosphate starvation coupled with changes in root growth and root architecture. In maize, we have identified two *Med12* genes, both of which are expressed throughout the plant. An Ac/Ds reverse genetic strategy was used to generate mutant alleles of *Med12a*; whereas that *Med12b* mutant allele was obtained through the UniformMu Transposon Resource. In this study, we measure root architectural traits to characterize the root system architecture and the phosphate response of maize *Med12* mutants. Preliminary results show that Zm*Med12* mutants present a root morphology typically associated with adaptation to low phosphorus, such as shallower roots and higher acid phosphatase activity.

Funding acknowledgement: CONACYT

**P332**

## Signatures of selection in sweet maize suggest a single donor shapes modern elite *se1* inbreds

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In the late 1970's a new mutation that confers high eating quality in sweet maize was characterized. This *sugaryenhancer1 (se1)* gene was recently fine mapped. With this information we are now able to examine the the history of the integration of this major gene in to the sweet maize germplasm. The *se1* allele was originally reported in a single inbred line, Ill677a, and it is thought to have penetrated a large breeding pool from this single donor. Here, we examine the history of this allele in a modern population, and the impacts of a single inbred in the background of a breeding pool. To explore the source and history of *se1* in modern sweet maize we compare 134 publicly available sweet maize lines in the Ames panel, to 50 modern high quality inbreds from the University of Wisconsin-Madison sweet maize breeding program which were genotyped using GBS, and imputed with FILLIN. Across the genome, scans of allele and haplotype frequency changes were done using Fst and IHS. An evaluation of imputation on this previously undescribed population suggests the current tools effectively impute this material. Also, we identified a single longer than expected haplotype surrounding the *se1* locus that matched the Ill677a haplotye, supporting a single introduction theory. Unexpectedly, there are many regions of extended Ill677a haplotype throughout the genome indicating multiple points of selection from this donor genome. A previous target of selection, *sugary1 (su1)*, also has an extended haplotype with low diversity throughout all of the sweet maize germplasm. Here we have confirmed the source of the *se1* allele in a modern commercial pool of germplasm, and provided an example of the integration of important line into breeding programs.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P333**

## Simple and quick method for GBS genotype imputation in bi-parental populations for linkage map construction in maize

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Genotyping-by-sequencing (GBS) is a relative newly and widely used genotyping method based on next-generation sequencing for diverse genetic applications (diversity analysis, QTL mapping, genome-wide association study and genomic selection). It is known for the advantages of time-saving, straight forward, cost-effective and high-throughput, however, also with the disadvantages of high missing, not well detection for heterozygosity and sequence error. To avoid the overestimation of recombination rate in linkage map construction for bi-parental population, we developed one method called “block imputation” to impute the high density genotype of bi-parental population and it was coded in R software. The method was future evaluated by using 955,690 SNPs generated by GBS for different types of maize genetic population, such as RIL, F2, DH, and BC1. It showed that the imputed genotype with the method could easily build a very good linkage map with different parameters for different population types. The flowering time and plant height of those populations were used to evaluate the linkage map, it showed that QTLs detected in this study were also detected in previous studies. Those results indicated that the method developed in this study is a powerful tool for GBS imputation in bi-parental populations; it also can be widely used in other high density genotyping platform.

Funding acknowledgement: Bill & Melinda Gates Foundation

**P334**

## Synergy between root hydrotropic response and root biomass enhances grain yield in maize (*Zea mays* L.)

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Roots of higher plants change their growth direction in response to moisture, avoiding drought and gaining maximum advantage for growth. There have been few studies of hydrotropism in crop plants, particularly on maize. Our goal was to test whether a robust hydrotropic response of maize roots was correlated to a better adaptation to drought and partial/lateral irrigation in field studies. We developed a laboratory bioassay for testing hydrotropism in primary roots of 233 maize elite hybrid DTMA hybrid lines (Drought Tolerant Maize for Africa). Once categorized in the laboratory, selected plants were tested in the field for their response to drought and partial lateral irrigation. The hydrotropic response was classified as robust or weak according to the angle of curvature, having a weak response those roots with less than 40°, and a robust response those roots with more than 40°. Selected robust and weak lines were analyzed in the field under three different irrigations: normal, partial lateral and drought. We found that there is a significant correlation between the root biomass of maize lines with robust hydrotropic response and grain yield. The phenotypic variation within the DTMA hybrid collection lead us to perform GWAS and to identify SNPs associated to robust hydrotropic response. Validation of the candidate genes throughout linkage analysis, expression, and DNA resequencing of plants with contrasting phenotypes is underway and these will help to determine the genetic network implied in the hydrotropic response. Our results indicate that hydrotropism participates in the control of root system development and that selection for robust hydrotropism might be a promising breeding strategy by marker-assisted selection for high yielding maize in drought prone environments.

Funding acknowledgement: DEGAPA, UNAM IG200515; CONACYT 177107; and MasAgro-SAGARPA (IMIC-2012-035)

**P335**

## Temporal Monitoring in Controlled and Field Environments Reveals Dynamic Genetic Factors Underlying Plant Height in the C4 Model Grass *Setaria*

(submitted by Max Feldman <mfeldman@danforthcenter.org>)
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Plant phenotypes, the morphological and physiological characteristics observed within populations, are influenced by both genetic and environmental factors. Plant height is one of the most influential components of plant architecture, and is profoundly influenced by growth environment. For plants, investment in vertical growth is a trade off. Vertical growth increases access to solar radiation but requires additional energy allocation for construction of stem material, amplifies cost associated with hydraulic transport and increases plant susceptibility to lodging. The primary goal of our studies have been to identify the major genetic loci that contribute to the variation of plant height within two independent genetically structured populations of *Setaria* sp. and how such phenotypes change temporally in response to environmental variables (water availability and planting density). To assess how genetics and environment interact to determine plant height in *Setaria* we have performed seven growouts of a interspecific *S. italica x S. viridis* recombinant inbred line population in field and controlled environment settings in addition to two grow outs of a *S. viridis* natural diversity panel in the Bellweather Phenotyping Facility at Donald Danforth Plant Science Center. Our studies identified several major genetic loci associated with variation in plant height across all experimental treatments and locations. However, the contribution of these loci differs depending on environment and developmental time. Our efforts focused on high-throughput phenotyping and haplotype map generation are also discussed.

Funding acknowledgement: Department of Energy (DOE)

**P336**

## The Effect of Artificial Selection on Phenotypic Plasticity: The Genotype by Environment Interaction Project in Maize

(submitted by Natalia de Leon <ndeleongatti@wisc.edu>)
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Around the world, crop species continue to attain remarkable productivity levels through the process of artificial selection and adaptation to modern agronomic practices. At the same time, this form of accelerated evolution is expected to reduce genetic variation in those regions undergoing stringent selection for enhanced productivity. The goal of this work is to determine whether the reduction in genetic variability on regions controlling productivity have also affected the ability of such improved cultivars to consistently maintain productivity across changing environmental conditions. A deeper understanding of the types of genetic architecture and modulation mechanisms controlling phenotypic plasticity, or genotype by environment (G X E) interaction, are expected to enhance our ability to predict performance of improved crop varieties under ever-changing environmental conditions. This evaluation utilized the framework of the Genomes to Fields (G2F) G X E Maize project to assess the effect of selection on standing variation and its implications in terms of variability associated with G X E. In 2014, the G X E Maize project evaluated a collection of 853 diverse maize hybrids across 22 locations in North America. Genotypes were evaluated for relevant phenological and agronomic characteristics. Climatic information was also collected in all locations. Genomic regions highly affected by selection explain a small percentage of the G X E interaction variability compared to regions not differentially affected by selection in traits such as plant height and grain yield. This presentation will focus on describing details related to these findings and a general description of the genetic architecture of G X E in this collection of materials.

Funding acknowledgement: Iowa Corn Promotion Board, National Corn Growers Association

**P337**

## The Effect of Crossing Strategy on Genomic Prediction in Maize

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Genomic Prediction of maize hybrid performance is becoming an increasingly important component of the maize breeding industry. Selection involves identification of improved inbred lines and identification of inbred combinations that produce the best hybrids. The goal of this study is to evaluate the value of producing new hybrids of candidate lines early in the selection process, as opposed to first identifying improved inbreds using testcrosses and subsequently identifying hybrid combinations utilizing genomic prediction. An Iodent type synthetic from six founder lines (PH207, PHG29, PHG35, PHG50, PHG72, and PHN11) and a Stiff Stalk type synthetic from six founder lines (B73, B84, LH145, NKH8431, PHB47, and PHJ40) were used as the progenitor populations to produce doubled haploid lines for this study. The doubled haploids were genotyped using the Illumina MaizeSNP50 genotyping array. The Iodent DH lines were testcrossed to PHB47, Stiff Stalk DH lines were testcrossed to PHG72, and DH lines were randomly paired between the groups to produce hybrids. Hybrids were evaluated at two locations in WI in 2014 and 2015. The primary trait used in the prediction evaluation was an index of yield and moisture, while 8 other ancillary traits were also evaluated. The intra-population phenotypic variance for the selection index was highest for the randomly paired population, followed by the Stiff stalk testcrosses, then the Iodents, which reflects the genetic variance within these populations closely. Predicted hybrids will be evaluated in 2016 and 2017, and should provide a clear picture of the utility of random experimental crosses have compared to test crossing.

Funding acknowledgement: Agreliant Genetics, LLC

**P338**

# The genetic architecture of leaf number and its genetic relationship to flowering time in maize

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The number of leaves and their distributions on plants are critical factors determining plant architecture in maize, and leaf number is frequently used as a measure of flowering time, a trait that is key to local environmental adaptation.
Here, using a large set of 866 maize-teosinte BC2S3 recombinant inbred lines (RILs) genotyped by using 19,838 SNP markers, we conducted a comprehensive genetic dissection to assess the genetic architecture of leaf number and its genetic relationship to flowering time.
We demonstrated that the two components of total leaf number (TLN), the number of leaves above (LA) and below (LB) the primary ear, were under relatively independent genetic control and might be subject to differential directional selection during maize domestication and improvement. Furthermore, we revealed that flowering time and leaf number are commonly regulated at a moderate level. The pleiotropy of the genes *ZCN8*, *dlf1* and *ZmCCT* on leaf number and flowering time were validated by NIL analysis. Through fine mapping, *qLA1-1*, a major-effect locus that specifically affects LA, was delimited to a region with severe recombination suppression derived from teosinte.
This study provides important insights into the genetic basis of traits affecting plant architecture and adaptation. The genetic independence between LA an LB enables optimizing leaf number for ideal plant architecture breeding in maize.

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

**P339**

# The genetics of just right: dose-response curve fits to drought and nitrogen limitation applied together allow mapping of loci that exhibit synergistic and antagonistic responses to stress combinations

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Crop improvement must accelerate with the increased human population and environmental changes; genotype prediction with the knowledge of genetic architecture and polygenic traits can shorten breeding cycle times. Incorporating climate prediction and adding information about genetic architecture to breeding programs can optimize the use of available resources. We analyzed the genetic architecture of 3D response curves for maize responses to abiotic stress mixtures—specifically, for response to drought and nitrogen deprivation in combination. Plant growth was measured in a Zea mays inter-mated recombinant inbred population exposed to carefully designed combinations of drought and nitrogen. Quadratic dose-response curves were fit globally and by polymorphic allele to determine which markers were associated with different dose-response curve shapes. Loci controlling dose response curve shapes exhibited non-linear effects, with the better-performing allele at mid-range combined stress typically different than the allele that provided best performance in extreme conditions.
To move toward mechanistic modeling of the QTL effects on the abiotic stress sensing and response system in plants, we developed a predictive function that models the sub-network responsible. The nonlinear function has two components, each of which integrates information on the levels of water and nitrogen available to the plant. By tuning the five parameters that control the shape and position of the function values, we reproduced the response surfaces we observed experimentally. Moreover, we were able to identify parameter combinations that produced a wide range of more desirable phenotypes, including surfaces that would predict better growth under very stressed conditions. We encourage application of our findings to crop protection mixture experiments or abiotic-biotic mixture experiments in elite germplasm.

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

**P340**

## The Genetics of leaf flecking in maize and its relationship to the defense response and broad-spectrum disease resistance

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Physiological/genetic leaf spotting, or flecking, is a mild lesion phenotype observed on the leaves of several commonly used maize inbred lines and has been anecdotally linked to enhanced broad-spectrum disease resistance. Flecking was assessed in the maize nested association mapping (NAM) population, comprising 4998 recombinant inbred lines from 25 bi-parental families, and in an association population comprised of 279 diverse maize inbreds. Joint linkage analysis was conducted with 7386 markers in the NAM population, and genome-wide association tests were performed with 26.5 million SNPs in NAM and with 246,497 SNPs in the association panel, resulting in the identification of 18 and one associated loci, respectively. Many of the candidate genes co-localizing with associated SNPs are similar to genes that function in plant defense response via cell wall modification, salicylic acid and jasmonic acid-dependent pathways, redox homeostasis, stress response and vesicle trafficking/remodeling. Significant positive correlations were found between increased flecking, stronger defense response and increased disease resistance, increased pest resistance and reduced yield. Mild flecking appeared to be associated with increased disease resistance but not with yield loss, suggesting that this phenotype could be used as a selection criterion for breeding programs trying to incorporate broad-spectrum disease resistance.

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

**P341**

## The role of teosinte (Zea mays spp. mexicana) introgression as a driver of stem pubescence in Mexican highland maize

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The cultivated maize landraces ( *Zea mays* ssp. *mays* ) and wild teosinte ( *Zea mays* spp. *mexicana* ) of the central highlands of Mexico are both characterized by marked stem pubescence. In contrast, the stems of both lowland maize and teosinte (*Zea mays* ssp. *parviglumis* ) are glabrous, suggesting that increased pubescence represents an adaptation to the highland niche. Molecular analyses have demonstrated the Mexican highland landraces carry a significant proportion of their genome from *mexicana* teosinte, presumably derived by introgression following domestication from lowland *parviglumis* , the ancestor of cultivated maize. It has been hypothesized that gene flow from highland teosinte was an important factor in the adaptation of cultivated maize to the highland niche.
In this work, we will test the hypothesis that the pubescence phenotype of Mexican highland maize is linked to regions of introgression from *mexicana* teosinte. Of all the Mexican highland races, Palomero Toluqueño (PT) shows the highest level of *mexicana* introgression (15-20%). With the availability of high-density molecular markers and whole genome sequence, it is possible map regions of introgression in the PT genome. In order to determine to what extent regions introgression co-localize with with the genes underlying pubescence, we have developed a population of 100 B73xPT BC1S5 recombinant inbred lines (RILs). We are evaluating RILs in both lowland and highland Mexican field sites, scoring a number of macrohair traits on both stems and leaves. We will present a description of macrohairs in the B73 and PT parents and report the range of phenotypes observed in the RIL population.

Funding acknowledgement: Cinvestav, CONACYT

**P342**

## The search for modifiers of the maize gametophyte factor (Ga1-S).

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Cross contamination of organic maize and landraces is a major concern as hybrids are selected for their production capabilities; leading to the loss of genetic diversity and quality traits possessed by the landraces. The maize gametophyte factor (Ga1) offers a solution to this problem. Ga1-S (S refers to the strong allele) confers cross incompatibility because it blocks the ability of pollen without this factor (ga1) to fertilize a plant that possesses this factor. The gametophyte factor is located on the short arm of chromosome 4. As yet, Ga1-S has not been molecularly isolated. We have found that in some genetic backgrounds, heterozygous Ga1-S / ga1 offers stronger resistance to ga1 pollen than in others. Identification of modifier genes will definitively useful in understanding the molecular basis of this factor. Two maize lines have been identified that are polymorphic to B73 relative to Ga1-S resistance to ga1 pollen: Ky21 shows less resistance and M162w shows greater resistance. Both Ky21 and M162w are progenitors of Nested Associated Mapping populations and recombinant inbred lines (RILs) have already been developed and genotyped for both of these with B73. 200 RILs each of Ky21 and M162w were sown in summer of 2014 and resulting plants were crossed with pollen from plants homozygous for Ga1-S. The strength of Ga1-S in each F1 line was evaluated by pollinating with Rscm2 ga1 pollen the first day and allowing open pollination on second day. A strong effect of Ga1-S is indicated if the resulting ear has few or no blue kernels and a weak effect is ear being heavily contaminated with blue kernels. A standardized scale of contamination was established to score ears. Preliminary results show two QTL, one on chromosome 2 and the other on chromosome 4. The latter QTL does not appear to include the ga1 locus.

**P343**

## Three-Fourths of Maize Presence-Absence Variants (PAVs) are not in Linkage Disequilibrium (LD) with Nearby SNPs

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Genomic structural variation is increasingly being recognized as a major contributor to phenotypic variation across species. In plants structural variants contribute to traits including resistance to soybean cyst nematode, boron tolerance in barley, dwarfism in wheat, flowering time in both barley and wheat, and aluminum tolerance in maize. Presence-absence variants (PAV), a form of structural variation where a DNA sequence is present in one genome but entirely missing from another, has been found to contribute to a number of genetic diseases in humans. While the association between PAVs and phenotype is still being explored in plants with some examples, including flavor quality in strawberries and submergence tolerance in rice have been uncovered. We enabled global explorations of the association between PAVs and phenotypic variation by conducting RNA-sequencing on the 27 inbred founders of the maize Nested Association Mapping (NAM) population. This exploration identified thousands of putative expressed PAVs that are absent from B73, the maize reference. In collaboration with Roche-Nimblegen these putative PAVs were used to design a Zeanome sequence capture array. Sequence capture of the NAM founders using the Zeanome array identified ~12,000 genomic PAVs that are absent from B73. Characterization of these PAVs shows that many have similarities to characterized proteins. Using the Fluidigm BioMark Dynamic Array, we genotyped for a subset of the PAVs and genetically mapped them in the NAM RILs. Additionally, a subset of ~200 PAVs were genotyped and linkage disequilibrium (LD) mapped using 400k single nucleotide polymorphisms (SNPs) in the Goodman Association Panel. Fewer than a quarter of PAVs were found to be in LD with SNPs. The lack of LD suggests that the power of association studies that include only SNPs could be improved by the inclusion of PAV genotyping.

Funding acknowledgement: National Science Foundation (NSF)

**P344**

## Toward cloning a major QTL for flowering time on maize chromosome 3

(submitted by Silvio Salvi <silvio.salvi@unibo.it>)
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Flowering time is a complex trait important for crop adaptation to the local environment. A major quantitative trait locus (QTL) for flowering time and number of nodes (ND), *qVgt3.05*, was previously identified on chromosome 3, bin 3.05, in a maize introgression library (IL) population derived from the cross B73 x Gaspé Flint (recipient and donor genotypes, respectively. Salvi et al. 2011, BMC Plant Biol 11, 4). In this region, other major flowering time QTLs have previously been mapped. The aim of this study was to fine map and clone *qVgt3.05*. From the cross between B73 and its early-flowering nearly isogenic line 39-1-2-33 which carries a 17-cM Gaspé Flint introgression on bin 3.05, more than 3,000 F2 plants were derived, genotyped, and phenotyped for ND. QTL mapping placed *qVgt3.05* within a 0.3 cM interval. In this cross, *qVgt3.05* explained 56.6% of the phenotypic variance. For positional cloning, additional 7,500 F2 plants were genotyped with SSR markers flanking the QTL interval. One-hundred-ten F3 and/or F4 recombinant lines were derived and grouped into seven classes based on the type of recombination events around the QTL. Phenotypic and molecular analysis of these lines enabled to further narrow down *qVgt3.05* to a 400-kb interval. Two putative candidates, a MADS-box gene and a Squamosa binding protein-transcription factor gene, mapped within this region. We are currently validating the role of the two candidate genes using several approaches, including comparison of allelic sequences and quantitative profiles of gene expression between the two parental haplotypes B73 and Gaspé Flint.

**P345**

## Validating SNPs controlling maize grain yield and plant height in Texas hybrid testcrosses

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Texas, as the 12th largest producer of maize within the United States, has not increased grain yield like the Midwestern States has in past decades. Grain yield is a complex quantitative trait, which is positively correlated with plant height in southern and central Texas maize. In this study, we constructed three bi-parental linkage populations (527 lines) using five parents to validate three SNPs (QTV1, QTV2 and QTV3) identified in a previous genome-wide association(GWAS) study, each explaining 3%- 5% variation in testcross grain yield across a diversity panel under dryland and irrigated conditions; QTV2 also affected plant height and flowering time. Using KASP assays, we genotyped F3:4 plants and investigated plant height and ear height in the F4:5 lines per se in the winter nursery at Weslaco, TX, 2014. F3:4 testcross hybrid yield trials were evaluated on irrigated and drought lands at College Station, TX in 2015 summer. QTV1 and QTV3 were identified consistently affecting plant height and flag leaf height in the F3:4 progenies (239 lines) of the population Ki3/NC356 in the winter nursery (Weslaco, 2014) and the derived hybrid yield trials (College Station, 2015); the allele from NC356 at QTV3 also increased grain yield in the hybrid yield trials across both irrigated and dryland conditions. In addition, we detected that grain yield was positively correlated with plant height, flag leaf height and ear height in these testcross hybrid yield trials. Therefore, we can take advantage of selection index between plant height and grain yield to select good performing lines in the population Ki3/NC356. The limited population size and small allele effects might weaken statistical power to validate the SNPs' effects in other two linkage populations. We only detected the QTV1 allele from NC356 increasing plant height and flag leaf height in TX740/NC356 testcross hybrid yield trials (College Station, 2015).

Funding acknowledgement: United States Department of Agriculture (USDA), China Scholarship Council, TEXAS CORN PRODUCERS, Texas A&M Agrilife research

**P346**

## What controls yield in NAM and Ames maize hybrids?

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Recent advances in Next Generation Sequencing (NGS) have provided us with new tools to explore the maize genome and the genetic architecture underlying important quantitative traits. Using NGS information, progress has been made in understanding how some developmental traits are regulated (e.g., flowering time, plant height). However, challenges remain; very little is known about how these traits or more complex traits (i.e., yield) are genetically controlled in maize hybrids. The main objective of our study is to expand our understanding of the genetic architecture underlying quantitative trait loci variation in maize hybrids. We evaluated a set of hybrids created by crossing a subset of the US National Maize Inbred Collection (Ames) and a subset of the US Nested Association mapping population (NAM) - those whose flowering-time ranges were similar to B73 – with PHZ51 and/or PHB47. Nearly 3,000 hybrids were evaluated in ten environments across the US over three years, for a range of developmental traits, as well as yield. The inbred lines used were genotyped using GBS (Genotyping by Sequencing). Finally, we imputed HapMap v3.2 data (a reference higher density SNP set) onto the GBS results and hybrid genotypes were inferred by combining tester and inbred genotypes. We use a variance-component approach to look at what genomic annotations can help us understand yield and we compare our results to other traits like flowering time and plant height. In this poster we will present findings from this study.

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

**P347**

## Whole plant phenotyping of maize diversity lines in controlled water stressed environments

(submitted by Nathanael Ellis <nellis@danforthcenter.org>)
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In light of climate change and increasing global demand for food, efforts to improve crops grown in variable climate conditions is essential. One major limiting factor for maize yield is drought. Developing drought tolerant maize would benefit agriculture in several ways: resilience to drought conditions, increased sustainability, and by expanding where maize can be grown productively in resource limited areas. Our study focuses on quantifying and discovering both above- and below-ground traits contributing to water stress resilience in maize. To measure multiple whole-plant traits, we incorporate a variety of techniques in highly controlled water stress experiments. In an effort to capture the diversity of maize, we focused on characterizing all 27 nested association mapping (NAM) parents. Experiments were conducted using a tightly climate controlled LemnaTec Scanalyzer 3D imaging system, integrated with three imaging modalities, visible (RGB), near-infrared (NIR), and fluorescent (FLUOR). Drought stress conditions were defined by field capacity: 25%, 50%, 75% and 100%. Ten replicates for each treatment for each parental NAM line were prepared with the whole experiment replicated twice. During a ten day period spanning approximately V2-V4, plants were automatically imaged daily by RGB to measure plant morphology and by near-infrared to measure internal water distribution. On the tenth day additional measurements were made for shoots, including biomass, plant height, leaf number, chlorophyll fluorescence. Additionally, we excavated and imaged the root systems and used the Digital Imaging of Root Traits (DIRT) software to quantify more than 30 traits, including stem width, crown root numbers at each whorl, high-resolution analysis of an excised root, and shape features of the whole-root system. We hope to identify important traits contributing to water stress resilience, and to use the NAM and other populations to identify their genetic basis in the context of whole-plant function.

Funding acknowledgement: EPSCoR

**P348**

## A sequenced-indexed reverse genetics resource for maize

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The availability of a mutant line in which a single known gene has been disrupted gives biologists a powerful tool in understanding the action of that gene. Thus, sequenced-indexed collections of single insertions are critical resources for elucidating gene function in organisms with a sequenced genome. Our NSF-PGRP-funded project is generating and sequence-indexing a collection of *Ds* transposon insertions created by *Agrobacterium*-mediated transformation. We are using a cost-effective method that takes advantage of next-generation sequencing (NGS) technologies. Specifically, our goals for this project are to: (1) Assemble a set of 120 roughly equidistant *Ds** launching platforms carrying a GFP marker that allows simple visual selection of element transposition from any region of the genome and, thus, enables researchers to generate regional gene knock-out collections; (2) Sequence-index several thousand *Ds** insertion sites from dozens of model platforms by NGS of 3-dimensional DNA pools on an Illumina MiSeq platform and data deconvolution with our InsertionMapper pipeline tool; and (3) Place all relevant information in our web-searchable database of insertion site sequences (http://acdsinsertions.org) cross-referenced to stocks available from the Maize Genetics Stock Center.

At present, we have mapped more than 5,500 transposed *Ds** target sites to the reference B73 genome with the help of our 3-dimensional DNA pooling strategy. All the lines generated in this project are listed in our database http://acdsinsertions.org, and more than 3,300 of them have been already sent to the Maize Genetics Stock Center for distribution.

Funding acknowledgement: National Science Foundation (NSF)

**P349**

## Altered DNA accessibility in B73, W22 and mutant genotypes

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Epigenetics is the study of heritable changes in gene expression without changes in the underlying DNA sequence. Epigenetic modifications include DNA methylation, covalent histone modifications, nucleosome occupancy, and chromatin structure changes. The position of nucleosomes has been shown to be intrinsically determined by the DNA sequence itself. This intrinsic occupancy map has been computationally determined for maize as the nucleosome occupancy likelihood (NOL) map. Using nucleosome protection assays at 400 loci, we have demonstrated differential nucleosome occupancy patterns between the NOL and two commonly used inbred lines, B73 and W22. Because the NOL is dependent on sequence information, these differences suggest the role of trans acting factors in affecting DNA accessibility. Differences were also observed between B73 and W22 suggesting variation in DNA accessibility. Using the same assays we tested seven putative trans acting chromatin factors. Several of them had the ability to affect DNA accessibility in comparison to their wild type counterparts. This supports the hypothesis that these trans factors play a role in establishing or maintaining the DNA accessibility landscape. We also performed bisulfite sequencing at several of the altered loci to determine the effects of these DNA accessibility changes on DNA methylation.

Funding acknowledgement: National Science Foundation (NSF)

**P350**

## Analysis of the Composite Insertion —A Novel Structure Generated by Alternative Transposition

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Maize *Ac/Ds* transposable elements are well known for their impact on gene expression and genome structure. In standard transposition, a single *Ac* or *Ds* element is excised from one locus and inserted into a new site. In contrast, Alternative Transposition acts on the termini of two different nearby elements, such as *Ac*, *Ds*, or *fAc* (fractured*Ac*, an element with one end deleted). Depending on their orientations, the two transposon termini can undergo either Sister-Chromatid Transposition (SCT) or Reversed-Ends Transposition (RET). Previous work in our group showed that SCT and RET events can also generate novel structures called Composite Insertions (CIs). The alternative transposition induces the re-replication of Ac/fAc and their flanking regions during S phase; abortion of the re-replication produces double strand breaks, which can be repaired to generate CIs. The CIs are composed of Ac, fAc and their flanking regions. We are interested in detecting the impact of CIs on gene expression. In order to obtain additional examples of CI, we used a highly efficient visual selection based on a colorless progenitor allele *p1-wwB54*. The *p1-wwB54* allele has a deletion of the first two exons of *p1* and contains reversed *Ac/fAc* termini in the *p1* gene. Because *p1-wwB54* does not have a functional *p1* gene, red kernel pericarp sectors can only be produced by alternative transposition events, which activate expression of *p2*, a nearby *p1* paralog that is normally not expressed in kernel pericarp. Here we describe the structures of several different CIs, including their locations, sizes, and internal compositions that reflect the extent of DNA re-replication and mode of DSB repair. We also present a model of how the CIs may affect the expression of *p2*. Our results provide new insight into the mechanisms of genome rearrangement and gene expression induced by alternative transposition.

Funding acknowledgement: National Science Foundation (NSF)

**P351**

## Comprehensive characterization of the relationship between transposable elements and genic regions in maize

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Plant genomes are comprised of a mosaic of genes and repetitive elements, including transposons. Often we think about these genomic features as distinct, with different regulation and chromatin. However, there are frequent examples of genic-transposon interactions that can influence regulation of either genes or transposons. The location of maize transposons relative to genes was assessed for different families of transposons and differing length classes of transposons. Some families of transposons are enriched in, or near, genes while others are rarely located within genes. Interestingly, some families of transposons are particularly enriched near active genes, while others tend to be in genes with lower expression levels. These families were often distinguished by different patterns of chromatin modifications and small RNA levels. Transposons located within maize genes often contain chromatin modifications associated with silenced chromatin, but do not appear to create a barrier for transcriptional elongation. There was limited evidence that transposons within maize introns resulted in premature termination or alternative splicing. These results provide evidence for mechanisms that allow large plant genomes to tolerate transposon insertions with minimal disruption of gene function.

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

**P352**

## Determining the Sequences That Induce Hyperactive Transposition of *mPing*

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Transposable elements are repetitive sequences, which have the ability to move throughout the genome. These elements are very useful because they can be used as tools for mutagenesis and gene discovery. The focus of this study is, *mPing*, a 430-bp deletion derivative of the natural occurring *Ping* element from rice. It exhibits high transposition activity and can reach a high copy number in plants. In contrast, an artificial deletion derivative of the natural occurring *Pong* element that shares approximately 80% identity to *mPing* exhibits low transposition activity. The overall question we are trying to address is what sequences are important for *mPing*'s transposition? To answer this, we screened a library of mutagenized *mPing* elements and identified high and low activity mutants. The high activity mutant, *mmPing20*, has seven base changes from the original *mPing*. Interestingly, four of these mutations are found within 100 bp, at positions 303, 307, 313, and 375. Our hypothesis was that one, or a combination of these mutations, are enhancing *mPing*'s transposition activity. To further determine which of these bases is affecting transposition of *mPing*, we are making mutants with various combinations of these base changes. In order to test the transposition rate, we are performing yeast transposition assays. Based on these results, we hope to find clues about which regions are important for recruiting the transposase proteins and promoting formation of the transposition complex. Not only this, but determining the specific bases that affect transposition activity can be used to increase *mPing's* mutagenesis efficiency.

**P353**

## Developing *mPing*-based activation tags

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Transposable elements are mobile DNA sequences referred to as "jumping genes," that move from one location in the genome to another. *mPing*, a Miniature Inverted Repeat Transposable element discovered in rice, is being used for mutagenesis because it transposes at high-rates and has a preference for insertion near genes. Adding promoter sequences to *mPing* can cause transcriptional activation of genes it inserts near and reveal their function. This activation tagging approach can be used as a gain of function strategy to identify redundant or lethal genes. To determine the efficacy of *mPing*-based activation tagging, the transposition of two elements, *mmPing20B* and *mmPing20F*, containing enhancer sequences from soybean β-conglycinin and figwort mosaic virus respectively are being studied in yeast and Arabidopsis. Yeast transposition assays were performed to determine the excision rate of these activation tagging elements compared to *mPing*.
Previous experiments indicate that adding sequences to the *mPing* element decreases its transposition. To overcome this effect, we have added the enhancer sequences to *mmPing20*, a hyperactive version of *mPing*. Because the enhancers used are similar in size, we expect *mmPing20B* and *mmPing20F* to show similar rates of transposition. Based on our results in yeast, we expect these elements to transpose in plants at similar rates to *mPing*. To test transposition in plants, *Arabidopsis thaliana* was transformed with an *mmPing20F*-GFP reporter construct using an agrobacterium-mediated floral dip method. Plants homozygous for the *mmPing20F*-GFP reporter construct will be transformed with a *Pong* ORF1 and transposase expression construct. The resulting plants will then be screened for GFP expression. Plants with high rates of transposition can then be used to evaluate this new activation tagging system.

Funding acknowledgement: National Science Foundation (NSF)

**P354**

## Epigenetic regulation mediates transcriptional responses to abscisic acid in *Zea mays*

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As sedentary organisms, plants must respond to numerous environmental cues and abiotic stresses to survive and maintain productivity. This is particularly true for crop plants, like *Zea mays*, which are often grown under suboptimal conditions. Environmental responses are often mediated by changes in gene expression, and epigenetic regulation of multiple stress responsive genes is key to maintaining yield and agricultural success in *Zea mays*. MOP1 is an RNA-dependent RNA polymerase that plays a major role in one epigenetic regulatory pathway in maize. The predicted function of MOP1 suggests that a loss of MOP1 function should lead to release of gene silencing. Consistent with this, many genes in *mop1-1* mutants are upregulated. Contrary to the canonical prediction, many other genes, including some stress responsive genes, are downregulated. A predicted DNA demethylase, DNG103, is downregulated ~2-fold in *mop1-1* mutants, suggesting that a hypomethylated condition of stress responsive gene promoters might coordinate transcriptional regulation in maize abiotic stress responses. Using RT-PCR and sequencing, a transcript for *Dng103* was confirmed to be ~6.2 kb and is predicted to encode an HhH-GPD superfamily base excision DNA repair DNA glycolase protein that includes an RRM-fold domain. Interestingly, the *Dng103* putative promoter region carries a response element that is predicted to facilitate gene expression responses to abscisic acid (ABA), a hormone known to play a crucial role in mediating plant abiotic stress responses through genetic and epigenetic transcriptional regulation. An initial qRT-PCR analysis confirmed that *Dng103* is down regulated in *mop1-1* and suggested that it is ABA responsive. To further explore ABA-mediated regulatory mechanisms, *Dng103* expression levels were monitored in maturing embryos with and without ABA under different conditions. These experiments will elucidate the specific roles of MOP1 and DNG103 in the regulation of stress response in maize.

**P355**

## Exploring the black box of siRNA communication between maize endosperm and embryo using B-A translocation lines.

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The seeds of the angiosperms include two genetically and epigenetically distinct tissues: the endosperm and embryo. The epigenetic signals exchanged between the endosperm and embryo may be important for the regulation and heritability of transposon silencing in the later life stages of plants. Here we establish a system using B-A translocation line that carries *Muk*, a locus that can heritably silence *MuDR* transposons in maize. Non-concordant seeds in which *Muk* is present or absent in the embryo or endosperm have been established in order to determine the degree to which signals can be transferred from one of these tissues to the other. The methylation level of siRNA target sites, the expression of *mudrA*, the profile of transferred siRNA, and the heritability of the transposon silencing initiated by the transferred siRNA will be examined.

We gratefully acknowledge the maize stock center for providing B-A translocation seeds.

Funding acknowledgement: National Science Foundation (NSF)

**P356**

## Exploring the stress-induced, epigenetic-regulated genome stability and epigenomic variability in maize

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The great ability of plants to respond and adapt to environmental changes involves sophisticated responses of cellular physiology, gene regulation and genome remodelling. Many studies demonstrated the important role of epigenetic mechanisms, such as DNA methylation, chromatin modifications and small RNAs, in spatio-temporal gene expression changes during stress response and adaptation.
To address the role of stress-induced epigenetic gene and TE regulation in maize, we analyzed the effects of salt and drought stresses on whole genome transcriptional modulation both in B73 inbred line and the epiregulator mutant *rmr6* (PolIV mutant impaired in short interfering RNA biogenesis and in the RNA-directed DNA methylation pathway). The leaf complete transcriptome and the smallRNA populations were analyzed in the two genotypes and integrated with the genome-wide distribution of H3K4me3, H3K9ac and H3K27me3 histone modifications investigated by ChIP-Seq on B73 under drought stress condition.
Integrating RNA-Seq, sRNA-Seq and ChIP-Seq data we identified and validated a robust list of epigenetic targets that affects different stress-responsive, developmental and metabolic pathways and which complete characterization is underway. These epitargets will be further evaluated at transcriptional and epigenetic level in maize plants subject to repeated stress pressure.
Given the importance of chromatin modifications and RdDM pathway in maize genome plasticity and in transcriptional silencing transposable elements, we are also investigating the identified target in *hda108* (a histone deacetylases actively involved in modulating gene expression and repeats silencing during plant development/reproduction) mutants. This will allow inferring the hierarchical relationship between these two epigenetic pathways. To do this we are analyzing total RNA and small RNA populations in the two mutants
Preliminary results indicated that about fifty genomic loci are miss-regulated in both mutants. These loci mainly comprise both transposable/repeated elements and important transcription factors involved in regulations of maize development and flowering time regulation

**P357**

## Genome wide analysis of small RNAs reveals dynamic changes during vegetative phase change in maize

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The transition from juvenile to adult vegetative growth is an important stage in plant development. Our previous work using the *MuDR* and *Mu killer* system has shown that the silencing of *MuDR* transposon is alleviated during the phase change, and this is associated with the transient loss of *lbl1/SGS3* expression, a key component in *trans*-acting small RNA pathway. In this study, we dissected the different developmental stages of the transition leaf, and found that the down-regulation of *lbl1/SGS3* was only observed at a specific stage during development of transition leaves. Further analysis of whole genome small RNA transcriptome data at this particular stage in the development of transition leaves demonstrates that small RNAs from transposable elements, *PHAS* loci, and inverted repeats are dramatically down-regulated, and that small RNAs mapped to DNA transposons are more extensively down-regulated than those that match with retrotransposons. Both up- and down-regulation of microRNAs and small RNAs involved in natural antisense transcripts were detected during the phase change. Finally, we also observed that although the changes of the transition leaf and *lbl1/SGS3* mutant overlap, large differences are observed, suggesting more genes may be participated in this important stage of plant development. Our data at whole genome level combined with a specific case reveals dynamic and complex changes in epigenetic regulation of transposons and other genomic components during maize vegetative phase change.This text is not italicized. *This text is italicized.* This text is not italicized.

Funding acknowledgement: National Science Foundation (NSF)

**P358**

## Genome-wide analysis of RNA Polymerase II occupancy in *Zea mays*

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RNA Polymerase II (Pol II) is generally considered to be the predominant polymerase for transcribing coding RNAs in eukaryotes, while noncoding RNAs are thought to be primarily transcribed by RNA Polymerases IV and V in plants. In RNA mediated gene silencing, these noncoding RNAs act as siRNA precursors and scaffolding molecules for epigenetic modifiers that induce gene silencing. Silencing is anticipated to result from the reduced activity, or altogether exclusion, of Pol II, but the dynamics of Pol II occupancy and activity during gene silencing have not yet been well described. A key component in an RNA mediated gene silencing pathway in maize is a protein called Mediator of paramutation1 (MOP1). In *mop1-1* mutants, silencing of some epigenetically regulated genes, like *BI*, is released, meaning that Pol II activity can be detected. It is not known if Pol II occupancy changes at regulated loci when silencing is occurring or released. By observing Pol II occupancy in *mop1-1* mutants, we can determine how loss of MOP1 impacts Pol II distribution. To address this, Pol II ChIP-seq is being used to determine Pol II occupancy genome-wide in wild-type and *mop1-1* plants. This data will also be correlated with genome-wide methylation and siRNA data to identify genome features relevant to gene silencing and MOP1 occupancy.

**P359**

## Genome-wide analysis of RNA polymerase-IV:Mop1 binding in *Zea mays*

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RNA-directed DNA-methylation (RdDM) is a plant-specific RNAi-like system that functions to conserve genome integrity through the transcriptional regulation of genes, transposable elements, retroviral insertions, transgenes, and other unstable genomic elements. This is achieved by *de-novo* DNA-methylation, histone modification, and higher-order chromatin structural changes mediated by RdDM, and these epigenetic modifications can be passed on to offspring. Genetic screens in maize (*Zea mays*) have identified loss-of-silencing phenotypes attributed to mutations in RNA polymerase-IV (RNAP4) and Mediator-of-Paramutation 1 (Mop1). Plant-specific RNAP4 plays a crucial role in RdDM through its transcription of coding and non-coding loci to generate small template RNA molecules which an RNA-dependent RNA polymerase (Mop1) uses to synthesize double stranded RNA (dsRNA) transcripts that are cleaved by Dicer-like proteins into single stranded 24-nucleotide small-interfering RNAs (siRNA). These siRNAs act as substrates for binding by Argonaute (AGO) proteins that hybridize with complementary nascent RNA polymerase-V transcripts to induce silencing via associated effectors. However, the genome-wide occupancy profile and underlying DNA sequences that are targeted by RNAP4 remain unknown. Recent evidence suggests that RdDM has complex and diverse roles in maize. With its large genome size and high level of epigenetic complexity, using maize as a model will allow us to answer remaining questions and define the roles of RNAP4 and Mop1 in RdDM using a genome-wide approach. Since Mop1 requires RNAP4 for siRNA biogenesis and physically interacts with RNAP4 but not RNA polymerase-II or V, we will utilize transgenic maize lines carrying an epitope-tagged FLAG:MOP1 fusion protein to perform chromatin-immunoprecipitation and high-throughput sequencing (ChIP-seq). Bioinformatics software will be used to identify loci that are targeted by RNAP4:Mop1 as well as other features of RNAP4 binding. This information will increase our understanding of the role and complexity of this important epigenetic regulatory system.

Funding acknowledgement: National Science Foundation (NSF)

**P360**

## GROseq and RNAseq comparisons identify RNA stability differences among seedling-expressed genes

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Gene expression is controlled at transcriptional, co-transcriptional, and post-transcriptional levels, yet the contribution of each to RNA abundance is often unknown. In plants, heritable changes to these levels of control can be influenced by poorly understood action(s) of Pol II-related DNA-dependent RNA polymerase (RNAP) complexes[1,2]. At least five types of these alternative RNAPs are found in grasses[3] but their functional significance remains unclear. Evaluating global run-on sequence (GROseq) profiles from 8-day maize seedlings, we found RNAP IV affects Pol II transcription at all 5' and 3' gene boundaries and within the gene body at a discrete set of 183 genes[4], some of whose dysregulation may contribute to developmental defects[5]. RNAseq profiles generated in tandem with the GROseq experiments and subsequently analyzed with counterpart GROseq profiles allowed comparisons to be made between nascent and total RNAs present in identical biological sources. Of the 180 RNAP IV-affected gene models identified with sense-strand GROseq reads, only 73 of these were also identified as RNAP IV-affected in RNAseq comparisons. Conversely, 222 RNAP IV-affected gene models were uniquely identified by RNAseq profiles. Preliminary differential coverage analysis (DESeq[6]) of GROseq vs RNAseq normalized read abundances indicates the two measures are significantly different for nearly 1/3 of all genes, consistent with the expectation that differences in RNA stability play a large role in defining mRNA levels[7]. GO-term analyses (g:Profiler[8]) indicate highly stable RNAs encode metabolic, photosynthetic, and stress response proteins while unstable RNAs are enriched for those encoding either kinases or unannotated proteins. A larger-scale effort is proposed to further define the nascent transcriptional landscape of maize, identify potential cases of co- or post-transcriptional control, and use mutant analyses to understand how alternative RNAP complexes affect gene expression.

**Citations** | **1** Erhard *et al.* 2009 *Science* **323**, 1201 | **2** Giacopelli & Hollick 2015 *Plant Phys.* **168**,1226 | **3** Haag *et al.* 2014 *Cell Rep.* **9**, 378 | **4** Erhard *et al.* 2015 *Genetics* **199**, 1107 | **5** Parkinson *et al.* 2007 *Dev. Biol.* **308**, 462 | **6** Anders & Huber 2010 *Genome Biol.* **11**, R106 | **7** McKinlay *et al.* 2011 *G3* **1**, 549 | **8** Reimand *et al.* 2011 *NAR* **39**, W307

Funding acknowledgement: National Science Foundation (NSF)

**P361**

## Investigating the transposition mechanism of the MITE *mPing*

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The miniature inverted repeat transposable element (MITE) *mPing* is a deletion derivative of the autonomous *Ping* element (*PIF*/*Pong*/*Harbinger* superfamily), and was found to be actively transposing in rice. We are using a previously developed yeast transposition assay to investigate the mechanism by which *mPing* transposes. *mPing* excises precisely, restoring the insertion site to its original state following transposition. We investigated the underlying cause of this precise excision site repair by mutating the target site duplications (TSDs) that flank the element upon insertion. Analysis of the mutants in the yeast transposition assay showed that alteration of the TSDs decrease the efficiency of excision site repair. This indicates that these TSDs produce a region of microhomology that facilitates precise excision site repair. We are also investigating if transposition differs in haploid and diploid cells. This is interesting because research indicates that DNA transposons in plants are more active in haploid pollen cells. We are testing this in haploid and diploid strains of yeast with identical genomic *mPing* insertions. Additionally, we are investigating the effect of *mPing* copy number on transposition rate. We hypothesize that the addition of *mPing* elements may increase transposition rates by effectively increasing the concentration of the transposase substrate. This effect is important as it may relate to the bursts of transposition observed for MITEs. To test this, we have developed a diploid yeast strain with a single copy of *mPing*, and will compare its transposition rate to the strain with two copies.

Funding acknowledgement: National Science Foundation (NSF)

**P362**

## Mapping and genetic transmission of maternal rough endosperm (mre) mutants that display parent-of-origin effects in maize kernel development

(submitted by Mary Daliberti <m.daliberti@ufl.edu>)
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The size of the maize kernel is thought to be determined in part by epigenetic regulation, specifically imprinting within the triploid endosperm. Genomic imprinting is the differential expression of alleles depending on maternal or paternal inheritance. Mutations in imprinted genes critical for seed development is expected to show parent-of-origin effect inheritance patterns. We screened *rough endosperm* mutants from the UniformMu transposon-tagging population for parent-of-origin effects using reciprocal crosses between heterozygous mutant plants and inbred lines. When inherited through the female gametophyte, *maternal rough endosperm* (*mre* ) heterozygous seeds develop a rough, etched, or pitted endosperm surface regardless of pollen genotype. Map locations for three *mre* loci were initially identified with BC1 mapping populations using single nucleotide polymorphism (SNP) markers. These loci, designated *mre1* , *mre2* and *mre3* , were fine-mapped on chromosomes 4, 6, and 10, respectively. Flanking insertion-deletion markers were used to test transmission of each locus. All three *mre* mutants transmit fully through pollen and confer a normal kernel phenotype when fertilizing wild-type plants. The *mre* mutants also transmit fully through the female gametophyte, but the frequency of rough endosperm kernel phenotypes is well-below 50% for all three loci. These data suggests that *mre* mutants confer a maternal parent-of-origin effect on seed development, but that *mre* phenotypes have low expressivity.

Funding acknowledgement: United States Department of Agriculture (USDA)

**P363**

## Massive rolling-circle amplification of Helitrons in plant genomes

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*Helitrons* are unusual eukaryotic DNA transposons that can readily capture host sequences and are, thus, evolutionarily important. Earlier computational analysis of *Helitron*-encoded conserved protein domains led to the proposal that *Helitrons* amplified by a rolling circle replication (RCR) mechanism, similar to that of some prokaryotic transposons. However, evidence supporting this mode of *Helitron* replication is weak, at best. Here, we have analyzed *Helitron* distribution in 27 plant genomes using HelitronScanner, a novel computational tool used recently to uncover many new eukaryotic *Helitrons*. We find that *Helitrons* are often distributed in tandem arrays, as predicted by the RCR model. The number of repeats in one array can range from a handful to several hundreds. Rice centromeres, in particular, harbor high numbers of tandem *Helitrons*. We propose a RCR model that conforms to the present *Helitron* landscape of plant genomes. Our study provides the first strong evidence of *Helitron* RCR in eukaryotes.

Funding acknowledgement: National Science Foundation (NSF)

**P364**

## Methods for accurate quantification of LTR-Retrotransposons copy number using short-read sequence data: a case study in *Sorghum*

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Transposable elements (TEs) are endogenous mobile elements that are ubiquitous in eukaryotic genomes, and as such, their activation and transposition serves as a powerful mutagenic force driving genome diversity and divergence. Comparative re-sequencing projects using short-read data have contributed tremendously to our understanding of genetic diversity, particularly in the gene space, among related taxa; however, quantitative comparative analyses of the repetitive content remains challenging. To overcome this difficulty, we have developed a robust method that estimates TE copy number from short-read sequences. To test the accuracy of this method, we first performed an *in silico* analysis of the reference *Sorghum bicolor* genome, resulting in copy number estimates that are strikingly statistically similar to the actual TE copy numbers. We then tested our method on real short-read data by estimating TE copy numbers in several accessions of *S. bicolor* and its close relative *S. propinquum*. Copy number estimation was performed using both reference-based and *de novo* methods for comparison. Both methods rank the families in similar order from highest to lowest abundance; however, the estimated copy numbers via *de novo* analysis differ significantly from that of the reference-based approach, particularly with respect to the age of the TE family. We find that *de novo* approaches are effective in estimating copy numbers for relatively young families, but underestimate the abundance of older families. In addition, interspecific reference-based mapping of *S. propinquum* reads to the *S. bicolor* database failed to efficiently describe TE content in *S. propinquum*, indicative of very recent TE activity leading to significant changes in their respective repetitive landscapes over very short evolutionary timescales. We conclude that reference based analyses are more suitable for estimating within-species variation while *de novo* approaches, though less accurate, are more reliable for evolutionarily distant comparisons.

Funding acknowledgement: WVU Advanced Energy Initiative

**P365**

## Over 8,000 new maize mutants in Release #8 from Uniform Mu

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The UniformMu population continues to provide new maize mutants free of charge for reverse genetics and functional genomics. More than 8,000 new mutants are now available through release #8, bringing the UniformMu total to over 75,000 germinal transposon insertions. In addition, availability of the W22 genome sequence has allowed UniformMu insertion sites to be re-mapped onto its own scaffold. This resulted in altered locations for 10% of the total UniformMu insertions and also led to identification of 4,030 (6%) new insertions. Mutant lines in UniformMu can be searched by gene sequence or by GRMZM maize-gene identifiers at MaizeGDB.org. Seeds can then be requested free of charge through the same site or directly from the Maize Genetics Cooperation Stock Center. The mutants are available in 14,020 independent seed stocks representing close to half of the maize filtered gene set (43%). Many (65%) of these genes have two or more mutant alleles. The UniformMu population was created by introgressing an active Mutator transposase into a W22 inbred to provide a uniform phenotypic background for mutant comparisons. Each UniformMu line carries 10 unique, germinal insertions. These lines are provided as segregating F3 material in a “Mu-off”, stable state with a mutable *bronze1* allele that serves as a visual marker for activity of the MuDR transposase. MaizeGDB.org provides UniformMu resource methods and tips for users, including information on how to request seed stocks and effectively test genotype-phenotype cosegregation.

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

**P366**

# Parent-of-origin effect mutants regulating endosperm cellular development in the maize seed

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Genomic imprinting in plants is an epigenetic phenomenon by which a subset of genes is expressed in a parent-of-origin–dependent manner. Although many maize imprinted genes have been identified through transcriptome analysis, imprinted genes with developmental functions in the maize seed have not been identified. We screened 178 *rough endosperm* ( *rgh* ) mutants for parent-of-origin effects using reciprocal crosses to inbred parents. Six *maternal rough endosperm* ( *mre* ) and three *paternal rough endosperm* ( *pre* ) mutants were identified. Characterization of the maternal-effect isolates shows a range of seed defects with several mutants showing embryo defects in addition to the endosperm phenotype. The *pre* mutants show a high frequency of embryo abortion leading to low oil levels in mature kernels. Embryo abortion is less frequent in *mre1* , *mre2* , *mre3* , and *mre-1014* suggesting these loci control seed size. Developmental sections of *mre1* suggest these mutants show a general delay in endosperm development with smaller starchy endosperm cells, delayed basal endosperm transfer cell layer development, and delayed accumulation of starch granules. The *mre2* mutant shows multiple starchy cell differentiation defects, while *mre3* mutants have an endosperm phenotype consistent with reduced sink strength. The quantitative RT-PCR of several endosperm specific transcripts is altered in *mre1* , *mre2* and *mre3* mutants compared to their wild type sibling seeds. Molecular mapping experiments identified four loci on chromosomes 4, 6, and 10. Additional mutant alleles obtained from the UniformMu reverse genetics resources appear to identify *mre1* , *mre3* , and *pre1* as imprinted genes in maize.

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**P367**

## Proper ontogeny and maintenance of paramutant states in *Zea mays* is defined by *required to maintain repression3* function

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Specific *purple plant1* alleles undergo paramutation[1], a behavior resulting in heritable epigenetic changes in gene regulation. Only repressed paramutant states (*Pl´*) are transmitted from *Pl´ / Pl-Rh* heterozygotes in apparent violation of Mendel's law of segregation. Ethylmethane sulfonate-induced mutations isolated in a screen for loss of *Pl´* repression identify at least fifteen *required to maintain repression (rmr)* loci. All six RMR-type proteins identified to date are involved in DNA-dependent RNA polymerase IV (Pol IV)-mediated 24 nucleotide (24nt) RNA biogenesis and are presumably orthologous to components of an *Arabidopsis* RNA-directed DNA Methylation (RdDM) pathway involved in silencing transposons. Although the biological roles of these 24nt RNAs in maize remain unclear, loss of the shared Pol IVa and Pol IVb largest subunit leads to global changes in Pol II transcription and gene dysregulations[2,3] associated with specific developmental defects[4]. Here we characterize a recessive mutation (*rmr3-1*) defining the *rmr3* locus that is distinguished from all known *rmr* mutations by specifying a unique phenotype of short stature, delayed flowering, and progressive leaf necrosis. This *rmr3* defect also correlates with reduced levels of 24nt RNAs representing repetitious features in developing cobs. Molecular mapping aided by whole-genome sequence places *rmr3* in a 12Mbp interval on chromosome *2* containing 186 gene models, though none encode potentially orthologous RdDM proteins. Possible candidates affecting chromatin condensation or RNA polymerase assembly are considered. Cloning *rmr3* will identify a novel component of 24nt RNA biogenesis having a role in developmental gene control distinct from that of Pol IV.

Citations: **1** Hollick *et al*. 1995 *Genetics* **141**, 709 | **2** Erhard *et al*. 2015 *Genetics* **199**, 1107 | **3** Hale *et al*. 2009 *PLoS Genetics* **5**, e1000598. | **4** Parkinson *et al*. 2007 *Developmental Biology* **308**, 462

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**P368**

## Sample sequence analysis of grass genomes indicates frequent and repeated horizontal transfer of LTR-retrotransposons

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LTR-retrotransposons are one of the most important components of plant genomes. A few studies have compared their properties across multiple "fully sequenced" genomes, but this requirement for a genome assembly means that only a small number of taxa can currently be investigated. To demonstrate an approach for comprehensive comparative analysis of LTR-retrotransposons across thousands of species, we generated low-depth sample sequence data for 19 previously-uncharacterized genomes from the panicoid grass subfamily. From the analysis, we identified highly dynamic changes in the activity of numerous LTR-retrotransposon families. The three Gypsy families *Milt, Xilon-Diguus*, and *Grande* were found to be particularly variable, exhibiting very different compositions even among closely related species. Numerous cases of lineage-specific activation and extinctions of specific LTR-retrotransposon families were observed. Using 62 public plant genome sequences and the 19 panicoid sample sequences, we investigated the possible horizontal transfer of LTR-retrotransposons. We found that the genus *Oryza* has had many horizontal transfers with the panicoid species, including at least 24 separate horizontal transfers involving 11 *Oryza* species and 19 panicoid species. Among the 11 *Oryza* species, *Oryza* sativa indica exhibited an extraordinarily high frequency of horizontal transfer with the lineage including *Echinochloa haploclada*.

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**P369**

## Small RNA sequencing reveals novel regulatory diversity in maize

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Small RNAs (sRNAs) in plants, divided into three functional groups based on their sizes of 21, 22, or 24 nucleotides, are ubiquitous components for regulating growth and development and maintain genome integrity by their control of gene expression. Deep sequencing of small RNAs from teosinte, 36 diverse maize inbred lines, and 27 hybrids derived from some of these inbred lines demonstrates that genome-scale accumulation patterns of sRNAs exhibit both a strong genetic component and dramatic genetic diversity throughout the whole maize genome. Distinct patterns of variation are observed for sRNAs associated with DNA transposons, long terminal repeat (LTR)-retrotransposons, genes and inter-genic regions. Performing the same analyses on the new genome assembly for the PH207 inbred line found similar patterns as observed with the B73 reference genome, indicating they are robust to use of different high-quality genome sequences. Furthermore, sRNA sequencing of inbred lines revealed variation for genomic features not captured by SNPs, offering potential insights into the recent evolution of regulatory variation. Integration of sRNA and RNAseq data finds genomic regions within or surrounding genes and inter-genic features where retrotransposon-derived sRNA variation is correlated with mRNA expression (most often positively), as well as where they are independent from each another. Our results indicate that sRNAs could contribute another component to regulatory diversity in complex genomes of maize.

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**P370**

## sRNA from the female gametophyte impacts the epigenome at fertilization affecting seed survival

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Double fertilization resulting from diploid parents occurs when both the diploid central cell and haploid egg from the female gametophyte are fertilized by haploid sperm cells, giving rise to the triploid endosperm and diploid embryo, respectively. If parental genome dosage deviates from 2:1 maternal:paternal genome copies, such as with interploidy crosses, the endosperm fails in development and the embryo dies. Through quantification of terminal seed phenotypes resulting from paternal excess crosses, we have identified natural variation in seed phenotypes. We therefore hypothesized that parental epigenetic state contributes to the sensitivity of endosperm development to changes in parental genome dosage. We evaluated terminal seed phenotypes from diploid small RNA (sRNA) and methylation mutants in crosses to tetraploid pollen parents. *mop1-1/+* and *Mop2-1/+* heterozygotes had higher frequencies of seed lethality compared to wild type crosses. No significant differences were observed for mutants defective in DNA methylation. Further characterization of triploid *mop1-1/+/+* individuals and their progeny identified adult plant phenotypes not previously observed in any offspring resulting from either wild type or parental balanced or paternal excess crosses. This strongly suggests that sRNA from the female gametophyte present at the time of fertilization impact the epigenome of the sporophyte affecting seed survival and adult plant development following paternal excess crosses in maize. To evaluate RNA expression during seed development, seeds from *mop1-1/+* heterozygous ears and B73 crossed with tetraploid pollen were collected fourteen days after pollination. Paternal excess crosses with B73 and *mop1-1/+* heterozygotes indicate a global reduction in storage protein accumulation as compared to wild type crosses. This validates previous analysis in which terminal seeds that fail earlier in develop accumulate less storage proteins. Interestingly, wild type paternal excess crosses had lower storage protein accumulation than *mop1-1/+* heterozygotes in paternal excess crosses.

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**P371**

## The mysterious *Ufo1*: What we have learned from global analyses

(submitted by Kameron Wittmeyer <ktw5072@psu.edu>)
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*Unstable factor for orange1* (*Ufo1*) is a dominant mutation that has proven resilient to efforts of gene cloning. Presence of *Ufo1* causes the up regulation of various *pericarp color1* (*p1*) alleles, a transcription factor controlling pigment production in floral tissues. Several studies have linked the up regulation of *p1* by *Ufo1* to a reduction of the epigenetic marks DNA methylation and H3k9me2. Efforts to map the mutation have yielded a ~40Mb region around the centromere of chromosome 10 that is void of recombination. Transcriptomics studies of mRNA and sRNA have yielded valuable insights into the effects of *Ufo1* globally. Interestingly, genes involved in DNA replication are down regulated in *Ufo1*. Total sRNA populations indicate that *Ufo1* is not involved in sRNA biogenesis unlike many of the discovered mutants involved in regulating gene silencing thus far. These methods have been useful in characterizing *Ufo1,* however they have not narrowed the list of candidate genes in the mapping region to a manageable size. Additional sequencing data for the genome and transcriptome are being generated to help identify sequence polymorphisms in the mapping region.

Funding acknowledgement: National Science Foundation (NSF)

**P372**

## Trans-Generational Inheritance in Response to Macronutrient Stress in Barley and Maize

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Understanding the contribution of epigenetic variation to gain-due-to-selection in crop breeding is a prerequisite to updating crop breeding methods to reflect current epigenomic science. The key may be to distinguish between transmission of heritable, phenotype-determining epigenetic variation that was *induced* in a parental population in response to stress, a non-stochastic process, versus selection for *random* variation resulting from a mostly stochastic process. As one component of research addressing this, I am studying trans-generational inheritance following parental macronutrient (N, P, K) stress in barley and maize. A growing number of studies have described the phenomenon of trans-generational transmission of adaptation or mal-adaptation to stress in various mammalian and plant species, but there are very few studies with major crop species, and very few studies addressing trans-generational response to nutrient stress in plants. For example, a huge field of research addresses phosphorus (P) nutrient sensing and how plants respond intra-generationally to varying P levels. Yet there are no studies of trans-generational adaptation/mal-adaptation to P nutrient stress in any major crop species, and only one report to date with a non-cultivated species! I have completed three generations of study with barley, and for one treatment observed a dramatic impact on offspring performance under limiting macronutrients when parents were exposed to limiting macronutrients: an essential doubling of tiller number, grain yield and grain P per plant, as compared with appropriate controls. Here I will describe some initial results from my current studies with maize. Some hypotheses currently being tested include: (1) a fraction of variants induced in response to stress are dominant/additive, and thus may contribute to performance of an F1 hybrid where only the male is stressed; (2) that performance gain will be observed in both stressful and non-stressful environments; (3) that paramutation plays a role in transmission of adaptive epigenetic programming.

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**P373**

## Transcriptome Analysis of *Ufo1* Identify Epigenetically Impacted Genes

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The maize *pericarp color1* (*p1*) gene encodes an R2R3 Myb transcription factor required for accumulation of phlobaphenes. Alleles of *p1* are denoted by their unique expression patterns; *P1-rr* has red pericarp and red cob glumes, while *P1-wr* has white pericarp and red cob glumes. *P1* gene is epigenetically controlled by *Unstable factor for orange1 (Ufo1)* and *mediator of paramutation* (*mop1*). A dominant mutation *Ufo1-1* (*Ufo1-1E)* can reduce DNA methylation of *P1-wr* leading to ectopic pigmentation accumulation. Besides pigmentation enhancement, *Ufo1-1E* has other pleiotropic developmental defects such as plant bending, stunted growth etc. We have isolated a silent allele (*Ufo1-1S*) of *Ufo1-1E* which has wild-type pericarp phenotype. The RNA-seq analysis showed that most differential expressed genes in *Ufo1-1S* are similar to those in wild type segregant as compared to *Ufo1-1E*. However, as compared to wild-type segregant, a small sub-set of genes are up or down regulated in *Ufo1-1E* with a similar pattern found in *Ufo1-1S*. In order to study transgenerational impact of *Ufo1-1,* we are analyzing smRNA-seq data obtained from *Ufo1-1E, Ufo1-1S,* wild-type segregant and B73. A comparison between smRNA in B73 and wild-type segregant shows that genes associated with differentially expressed smRNA are enriched in stress response functions. Maize *mop1* is an RNA-dependent RNA polymerase (RdRP) which is involved in RNA-directed DNA methylation (RdDM) and is required for the majority of 24-nucleotide siRNA accumulation. We are comparing smRNA profiles from young cob libraries of *Ufo1-1, mop1-1*, and *Ufo1-1; mop1-1* double mutants to find similarities or differences between *mop1-1* and *Ufo1-1* epigenetic pathways. To address the question, if *Ufo1-1* and *mop1-1* may have transgenerational epigenetic impact, epigenetic recombinant inbred lines (epiRILs) of *Ufo1-1* and *mop1-1* are being developed.

Funding acknowledgement: NSF-MCB-Genetic Mechanisms award number 1051654

**P374**

## Transposable element evolution in the bambusoideae

(submitted by Domitille Chalopin <chalopin@uga.edu>)
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The number of genes in angiosperm genomes varies very little compared to the ~800-fold difference in their range of genome sizes. Analysis of the intergenic regions in angiosperms indicates that these variations, in most cases, can be explained by differences in the amplification and/or retention of transposable elements (TEs), especially LTR retrotransposons. Here, we investigate the evolution of TEs and their impact on genome size evolution in the bamboo lineage (Bambusoideae, Poaceae), which represents one of the major branches of the grasses. With a large number of species, a large range of genome sizes and differences in ploidy, the bamboo lineage is an ideal model to perform comparative analyses and investigate TE impact on plant genome evolution. We collected 56 species distributed over the three main tribes, estimated genome sizes by flow cytometry and generated low-depth sample sequence data. With determined genome sizes ranging from 650Mb (for the diploid R. distichophylla) to 4200Mb (for polyploid Eremitis species), we estimated TEs to account for at least 45-85% of bamboo genomes. The most abundant Copia and Gypsy retroelements were further investigated within and between species, indicating TE family distribution and divergence, as well as possible instances of horizontal DNA transfer. Together, the results allow us to better understand bamboo genome evolution and the history of bamboo speciation.

Funding acknowledgement: National Science Foundation (NSF), NIFA

**P375**

# Variation and Heritability in a Population of Epigenetic NILs

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The heritability of many phenotypes is not fully explained by genomic DNA sequence. Thus, I am exploring epigenetic variation as a possible source of the "missing heritability" of complex traits. In maize (*Zea mays*), 85% of the genome is transposable elements which contribute to epigenetic variation by chromatin remodeling, often via small RNAs (sRNAs) and DNA methylation. To study epigenetic regulation of phenotypic inheritance, an inbred population carrying *mediator of paramutation1* (*mop1*) was used. This recessive mutation results in a dysfunctional RNA-dependent RNA polymerase2 (RDR2), and therefore a global decrease in 24-nt sRNA molecules which are usually associated with the maintenance of transposon silencing. Exposing the genome to this condition may generate epigenetic variants in a nearly identical genetic population. In addition, *red fluorescent protein* (*RFP*) was introduced as a reporter gene fused to the tissue-specific promoter for *FLOURY2* (*FL2*) which encodes the abundant alpha-zein seed storage proteins. The population was grown in a nitrogen-deficient nursery to expose active nitrogen-utilization genes to altered regulation via epigenetic variation.
A screening in 2014 revealed phenotypic variation – a high frequency of variants in developmental pathways were seen and the previously stable intensity of RFP showed a wide range of expression following exposure to *mop1*. The heritability of these traits was tested and hybrids were created for 2016. Future work will also expand this system to utilize the Illinois Long Term Selection Lines. The results to date indicate that these populations will reveal insight into the epigenetic regulation of gene expression and the inheritance of this regulation.

Funding acknowledgement: United States Department of Agriculture (USDA)

# Late Poster Abstracts

**P376**

## Promoter deletion analysis of an invertase inhibitor during Arabidopsis seed development

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In Arabidopsis, endosperm development can be separated into two phases, the initial syncytial phase followed by the cellularized phase. Since embryo growth rates accelerate after endosperm cellularization, we investigated whether genes involved in nutrient supply are differentially expressed around the time of endosperm cellularization. We discovered that two invertase inhibitors are expressed in the embryo-surrounding region of the syncytial endosperm, and that they are down-regulated after endosperm cellularization. Invertase inhibitors suppress the activity of invertase, which converts sucrose to glucose and fructose to support growth and development. Our data indicated that invertase activity increases after endosperm cellularization to meet the embryo's growing demand for nutrients. Since endosperm cellularization requires a chromatin-remodeling complex containing the FIS2 protein, it's likely that FIS2 represses the expression of invertase inhibitors. To identify the cis-regulatory elements in the promoter region of one of the invertase inhibitors, we generated promoter deletion constructs to delete the 5' end of the 1 Kb promoter in 100 bp increments. If a positive cis-element is deleted we expect a decrease in promoter activity during the syncytial phase. However, if a negative cis-element is deleted then we expect an increase in promoter activity during the cellularized phase. This approach will allow us to determine the location and the nature of the cis-elements that regulate the transcription of invertase inhibitors. Moreover, we can determine whether there are cis-elements that recruit FIS2 to repress the expression of invertase inhibitors.

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