

# 61st Annual Maize Genetics Conference

Program and Abstracts



**March 14 – March 17, 2019**

Union Station  
St. Louis, Missouri, USA

## **This conference received financial support from:**

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*We thank these contributors for their generosity!*

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### **Cover image description**

Drying maize cobs at Manaslu track in Nepal

### **Cover art by**

Maïke Stam  
University of Amsterdam,  
Netherlands

## General Information

### Meeting Registration

Thursday: 3:00 PM to 9:30 PM: Depot Registration Office

Friday: 7:00AM to 1:30 PM: Depot Registration Office

### Meals

All meals will be served buffet style in the Midway; 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.

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

The maize meeting is a forum for presentation and discussion of unpublished material.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

### Hospitality

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

### Steering Committee

Please share your suggestions and comments about the meeting with the 2019 Steering Committee

Michael Muszynski, Chair.....(mgmuszyn@hawaii.edu)	Ex officio:
Clint Whipple, co-Chair..... (whipple@byu.edu)	Carson Andorf
Hilde Nelissen.....(hinel@psb.vib-ugent.be)	David Braun
Andrea Gallavotti.....(agallavotti@waksman.rutgers.edu)	Marty Sachs, Local Host
Andrea Eveland..... (AEveland@danforthcenter.org)	Alain Charcosset
Maike Stam ..... (M.E.Stam@uva.nl)	John Portwood
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Sylvia Sousa..... (sylvia.sousa@embrapa.br)	
Natalia de Leon..... (ndeleongatti@wisc.edu)	
Jeff Ross-Ibarra ..... (rossibarra@ucdavis.edu)	
Yongrui Wu ..... (yrwu@sibs.ac.cn)	
Todd Jones ..... (todd.j.jones@pioneer.com)	

### Acknowledgements

Many thanks go to John Portwood and Carson Andorf 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 the Union Station staff for their help in organizing this conference, and to Darwin Campbell and John Portwood for providing AV and other support. Thanks go to Thomas Slewinski and Todd Jones for their efforts in securing funding to support graduate student attendance at this meeting. Finally, many, many thanks go to Marty Sachs for his work as local organizer and for his wisdom in all things related to the Maize Meeting.

## From the Maize Genetics Executive Committee:

Chair: Jianming Yu 2019, Vice Chair: Natalia de Leon 2020, Shawn Kaeppler 2018, Patrick Schnable 2018, Kathy Newton 2019, Karen Koch 2020, David Jackson 2021, Ed Buckler 2022, Marilyn Warburton 2022, and the two new members: David Braun 2023, Ruth Wagner 2023.

### Awards:



**The Early Career Maize Genetics Award** will be given to an individual that has been in a permanent position for less than 8 years. It is expected that the awardee will have made significant research contributions through genetic studies of maize or related species. (See MaizeGDB).  
The 2019 Awardee is Andrea Gallavotti at the Waksman Institute, Rutgers University.



**The Mid-Career Maize Genetics Award** will be given to an individual that has been in a permanent position for 9-20 years. The winner will have an outstanding track record of discovery research in maize (or related species) genetics. (See MaizeGDB).  
The 2019 Awardee is Marja Timmermans at the University of Tübingen.



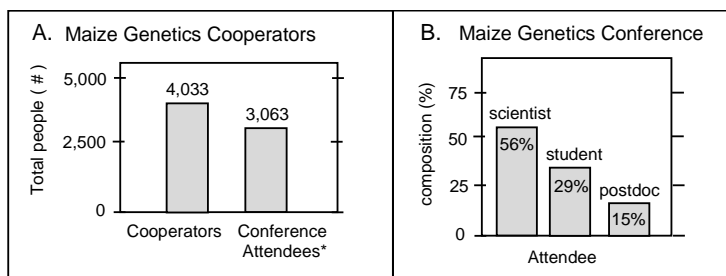
**The R.A. Emerson Award** recognizes individuals for their extraordinary lifetime achievements in maize genetics. Recipients of this award shall be leaders in the maize community who have made seminal contributions to our understanding of maize genetics. To be eligible for this award, the nominee should have held a permanent position for over 20 years. (See MaizeGDB)  
The 2019 Awardee is Gerry Neuffer at the University of Missouri, and this year Ed Coe will be presented with the 2018 award.



**The Barbara McClintock Prize for Plant Genetics and Genome Studies** has been created to memorialize the unequalled contributions of Dr. McClintock through providing recognition to the most outstanding plant geneticists of the present era. In memory of the many contributions of Dr. McClintock, this Prize will be awarded each year to one or more of the most creative minds and productive scientists in the study of plant genome structure, function and evolution, including the analysis of gene regulation and epigenetics. The 2019 Awardee is Detlef Weigel who will present the McClintock Prize Address. The 2020 Awardee will be announced at the meeting and will present the address next year (See MaizeGDB).



## Defining the maize genetics community: Who are we?



The Maize Genetics Community:  
**A.** Maize Genetics Cooperators. Total number is based on the e-mail database from MaizeGDB.org. The conference attendees include meetings held in the US from 2008 to 2017. Each attendee is counted only once during this time.  
**B.** Maize Genetics Conference attendee composition. Data are from 2012 and 2015.

## NSF-funded Research Coordination Network for maize genetics:

The National Science Foundation is supporting a 5-year Research Coordination Network project titled “Broadening and Energizing the Maize Research Community”. The project began in January, 2018, and is coordinated by the Maize Genetics Executive Committee. The grant funds activities at the Maize Genetics Conference including the MaGNET program and travel awards to increase disciplinary breadth and underrepresented participation. In addition, the funding allows the Maize Genetics Conference to systematically enrich the program during the term of the grant. Mid-year conferences are planned yearly to focus on specific topics that are important to the community. The first mid-year conference was held in Madison, WI in September 2019 and included an overall visioning session as well as focus on Functional Genomics Tools and Resources. Outcomes of the mid-year conferences will be provided to the community through white papers or other summary formats. Teams have been assembled within the RCN to focus on: Functional Genomics Tools and Resources; Informatics Tools, Resources, and Services; Training and Student Recruitment; Developing Country Interface and Community Breadth; and Industry Interface. We appreciate the support from the National Science Foundation for this initiative and are excited about the potential for the grant to substantially advance and transform our community.

## Data Management Made Simple

It is the responsibility of every Maize Researcher to make data from publicly funded research Findable, Accessible, Interoperable and Reusable (FAIR; [go-fair.org](http://go-fair.org)). Here we outline some basic guidelines for good data management. We are always happy to answer your questions on these issues! <https://www.maizegdb.org/contact>

### 1. Put your Data in the right Database.

Some examples: *DNA/RNA/Protein Sequences, genome assemblies* should go to NCBI, EBI or DDBJ: NCBI (US), EBI (Europe), and DDBJ (Asia) provide stable, long-term databases for DNA, RNA and protein sequence data and create stable identifiers (accessions) for datasets. These three share sequence data on a daily basis so data deposited at one is available at all. Each has multiple sub-databases, for example, NCBI has SRA and GEO for un-mapped and mapped sequence reads.

*SNPs*: All non-human SNPs should be submitted to EVA at EBI.

*Genome Assemblies*: Please submit genome assemblies to EBI or NCBI Genomes. We understand this can take some time to complete. We can help, don't be tempted to simply submit contigs to Genbank. If you are unsure where to submit data, or need help submitting, please ask anyone at MaizeGDB. If your journal article refers to data NOT published with your article, **please make sure to obtain and add a persistent identifier and location of your data in your article.**

### 2. Don't rename genes that already have names.

Renaming genes that already have names is a HUGE problem in maize, especially when an existing name is reused for a different gene. Please look up your gene at MaizeGDB before assigning a name, and follow the maize nomenclature guidelines. (<https://www.maizegdb.org/nomenclature>).

### 3. Attach complete and detailed metadata to your data sets, and use accepted file formats.

When you deposit data, you are asked for information about your data (metadata). Please give this the same careful attention you give to your bench work and analysis. Datasets that are not adequately described are not reusable or reproducible, and raise questions about the carefulness and accuracy of the research

### 4. Insure your data sets are "machine readable".

Computers can find data that matches a search query. Use complete, proper identifiers, including the proper case (LG1 is not the same as lg1), use permanent identifiers wherever possible, and include GO, PO, PATO terms when possible. Please check and validate that your data is in common, well-used machine readable formats.

### 5. Publish your data with your paper.

Sometimes data are too large to publish as a table or supplementary material with your paper. These data can be deposited in data repositories, which provide accessions or DOIs (stable identifiers). DOIs should be listed in your paper.

### 6. Budget time for Data Management.

Please budget time to do a good job of managing your data as you are with the other aspects of your research.

### 7. Familiarize yourself with the FAIR data sharing standards.

To support the reuse of scholarly data, a group of data scientists have created a set of recommendations to make data Findable, Accessible, Interoperable and Reusable. Here are some resources: <https://www.go-fair.org>, <https://doi.org/10.1093/database/bay088>.

The MaizeGDB team

## **Useful Links**

### **2019 Maize Meeting Website**

[http://maizegdb.org/maize\\_meeting/2019](http://maizegdb.org/maize_meeting/2019)

### **2020 Maize Meeting Website (Available November 2019)**

[http://maizegdb.org/maize\\_meeting/2020](http://maizegdb.org/maize_meeting/2020)

### **Abstract Book (Electronic version)**

[http://maizegdb.org/maize\\_meeting/abstracts/2019Program.pdf](http://maizegdb.org/maize_meeting/abstracts/2019Program.pdf)

### **Cover Image**

[http://maizegdb.org/maize\\_meeting/coverart/](http://maizegdb.org/maize_meeting/coverart/)



## The MaGNET Program and 2019 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.

### 2019 MaGNET Awardees

#### **Undergraduate**

Jelani Freeman, Florida A&M University	-----
Karen Granados, Iowa State University	Poster #166
Gabdiel E. Yulfo-Soto, Michigan State University	Poster #140
Jaydon Lynch, Montclair State University	Poster #11

#### **Graduate Student**

Dylan Oates, University of Hawaii at Manoa	Poster #95
Dakota Jackson, Michigan State University	Poster #266
Brianna Griffin, Iowa State University	Poster #219
Eli Huggis, Michigan State University	Poster #102

#### **Mentor Accompanying Student**

Gokhan Hacisalihoglu, Florida A&M University	Poster #116; 228
Angel Del Valle Echevarria, University of Hawaii	Poster #101



The MaGNET program of the Maize Genetics Conference is supported by grant IOS-1748978 from the National Science Foundation.



# Primarily Undergraduate Institutions and Disciplinary Breadth Awards

**Primarily Undergraduate Institutions (PUI) and Disciplinary Breadth (DB) are two new financial aid programs** that seek to recruit and retain scientists from PUIs and plant-related disciplines 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. The program also provides an opportunity for awardees to explore potential collaborations and develop career contacts.

Each award helps defray the cost of attending the Maize Genetics Conference, including registration, food, lodging and airfare. 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, and be either citizens or permanent residents of the USA. To help provide a more integrative and effective experience at the Conference for student awardees, faculty who accompany one or more eligible student applicants are also eligible to apply for a PUI or DB award.

## 2019 PUI Awardees

### **Student**

Katie Hillman, Hamline University	Poster #100
Christopher Morales Farlan, Hamline University	Poster #329
Rhea Sablani, Whitman College	Poster #220
Yuli Buckley, Whitman College	Poster #120
Rachel Calder, University of Washington	Poster #42; 78
Micah Rambo, Whitman College	Poster #174
Benjamin Cosgrove, Whitman College	Poster #51
AB (Abenezer) Abera, Hamilton College	Poster #150

### **Faculty**

Tyrell Carr, Saint Augustine's University	-----
Britney Moss, Whitman College	Poster #121
Thelma Madzima, University of Washington	Poster #376
Natalie Nannas, Hamilton College	Poster #258

## 2019 DB Awardees

### **Postdoc**

Kevin Lehner, Colorado State University	Poster #298
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### **Faculty**

Nhu Nguyen, University of Hawaii	Poster #154
Subbaiah Chalivendra, Louisiana State University	Poster #119



The PUI and DB programs of the Maize Genetics Conference are supported by grant IOS-1748978 from the National Science Foundation.

## SCHEDULE OF EVENTS

Talks will be held in the Grand Ballroom.  
Posters will be displayed in the Midway.

### Thursday, March 14

#### 8:00 AM – 1:30 PM **OPTIONAL CAREER WORKSHOP (PRE-REGISTRATION REQUIRED)**

8:00 AM – 1:30 PM **Careers in Science beyond Academics** Bayer Crop Science  
*Includes travel to/from Bayer, light breakfast, networking, tour of research facility, seminar by Dr. Rob Martienssen, and panel discussion.*

#### 1:00 PM – 6:00 PM **OPTIONAL PRE-CONFERENCE WORKSHOPS**

*All workshops will be located on the main level in Grand Ballroom C*

1:00 PM – 2:00 PM **MaizeMine** Grand Ballroom C

~~2:00 PM – 3:30 PM **Gene editing and UAV drones: Perspectives and connections to drive research in an evolving regulation landscape.** Grand Ballroom C~~  
*Workshop cancelled*

3:30 PM – 5:00 PM **Maize Epigenetics and Chromatin network: Maize EPIC** Grand Ballroom C

5:00 PM – 6:00 PM **Make your data FAIR - Next Generation Data Management** Grand Ballroom C

3:00 PM – 9:30 PM **REGISTRATION** (Depot Registration Office)

3:00 PM – 6:00 PM **POSTER HANGING** (Midway)

5:00 PM – 5:45 PM **MaGNET Awardees and Mentors Introductions** (Conductor Room)

6:00 PM – 7:00 PM **DINNER** (Midway)

#### 7:00 PM – 9:00 PM **SESSION 1 – WELCOME / COMMUNICATING WITHIN AND BETWEEN CELLS AND PLANTS**

Chair: Michael Muszynski / Hilde Nelissen Talks 1-5. Pages 31-35.

7:00 PM **WELCOME AND ANNOUNCEMENTS** (Grand Ballroom)

7:15 PM **Carolyn Rasmussen, University of California - Riverside** [T1]  
*Role of the microtubule-binding protein TANGLED1 in plant cell division and growth*

7:35 PM **China Lunde, University of California - Berkeley** [T2]  
*Tasselseed5 overexpresses a wound-inducible enzyme, ZmCYP94B1 that affects jasmonate catabolism, sex-determination, and plant architecture in maize*

- 7:55 PM **Fionn McLoughlin, Washington University - St. Louis** [T3]  
*Maize multi-omics reveal roles for autophagic recycling in amino acid, nucleotide and carbohydrate metabolism during carbon starvation*
- 8:15 PM **Matthew Warman, Oregon State University** [T4]  
*Pollen vegetative cell and sperm cell transcriptomes help predict mutation effects on fertilization success*
- 8:35 PM **Benjamin Julius, University of Missouri** [T5]  
*An “a-maizing” connection between cell wall biosynthesis and carbohydrate partitioning: Brittle Stalk 2-Like3 encodes carbohydrate partitioning defective28*
- 9:00 PM – 1:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY** (Midway)

## **Friday, March 15**

7:00 AM – 8:00 AM **BREAKFAST** (Midway)  
7:30 AM – 12:30 PM **REGISTRATION** (Depot Registration Office)

8:00 AM – 10:10 AM **SESSION 2 – EMERGING TOOLS AND CHALLENGES**  
Chair: Thomas Slewinski Talks 6-11. Pages 36-41.

8:00 AM **ANNOUNCEMENTS** (Grand Ballroom)

8:15 AM **Qiuyue Chen, University of Wisconsin - Madison** [T6]  
*TeoNAM: A nested association mapping population for domestication and agronomic trait analysis*

8:35 AM **Kathryn Michel, University of Wisconsin - Madison** [T7]  
*Combining ability, per se yield components, and GxE in the Stiff Stalk heterotic group dissected using new genome assemblies combined with exome-capture genotyping of a multi-parent population*

8:55 AM **Zhikai Liang, University of Nebraska - Lincoln** [T8]  
*Genome-Phenome Wide Association Study (GPWAS): Using high dimensional phenotype data to identify the genes that specify the traits of maize*

9:15 AM **Elizabeth Lee, University of Guelph** [T9]  
*Functional genetic diversity in the commercial germplasm pool – Is there anything left?*

9:35 AM **Ruth Wagner, Bayer Crop Science** [T10]  
*Sequence, assembly and annotation of Bayer Crop Science's maize inbred line LH244; A new resource for maize genetics and transformation*

9:55 AM **Lisa Harper, USDA-ARS** [T11]  
*Next generation data management.*

10:10 AM – 10:40 AM **BREAK**

10:40 AM – 12:30 PM **SESSION 3 – INVITED SPEAKERS**  
Chair: Clint Whipple Pages 26 & 27.

10:40 AM Introduction

10:50 AM **Zachary Lippman, Cold Spring Harbor Lab** [IS1]  
*Unveiling and harnessing mechanisms of epistasis and quantitative variation in plants*

11:40 PM **Sherry Flint-Garcia, USDA-ARS** [IS2]  
*The genetics and consequences of maize domestication and breeding*

## **Friday, March 15 (continued)**

12:30 PM – 1:30 PM     **LUNCH** (Midway)

1:30 PM – 4:30 PM     **POSTERSESSION 1** (Midway)

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 2:30 to 4:00 PM in Midway

4:40 PM – 6:00PM     **SESSION 4 – THE GENES THAT MAKE MAIZE I**  
Chair: Yongrui Wu     Talks 12-15. Pages 42-45.

4:40 PM     **Zhaobin Dong, University of California - Berkeley**     [T12]  
*The regulatory landscape of a core maize domestication module controlling bud dormancy and growth repress*

5:00 PM     **Li Chaobin, China Agricultural University**     [T13]  
*The ZmbZIP22 transcription factor regulates 27-kD  $\gamma$ -zein gene transcription during maize endosperm development*

5:20 PM     **Josh Strable, Cornell University**     [T14]  
*Formation of the maize blade-sheath boundary: evidence for a prepatter*

5:40 PM     **Clinton Whipple, Brigham Young University**     [T15]  
*Few branched1 is a positional regulator of inflorescence architecture in maize*

6:00 PM – 7:00 PM     **DINNER** (Midway)

7:00 PM – 9:00 PM     **SESSION 5 – AWARDS & MCCLINTOCK PRIZE PRESENTATION**  
Chair: Jianming Yu     Page 30.

7:00 PM     **Jianming Yu, MGEC Chair**  
*M. Rhoades Early-Career and L. Stadler Mid-Career Awards*

7:25 PM     **Natalia De Leon, MGEC Vice Chair**  
*R. Emerson Lifetime Awards 2018 and 2019*

7:55 PM     **Nathan Springer, University of Minnesota**  
*McClintock Prize Presentation*

8:10 PM     **Detlef Weigel, Max-Planck-Gesellschaft**  
*Epistasis, the spice of life: Lessons from the study of the plant immune system*

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

## **Saturday, March 16**

7:00 AM – 8:00 AM **BREAKFAST** (Midway)  
8:00 AM – 12:00 PM **REGISTRATION** (Depot Registration Office)

8:00 AM – 10:00 AM **SESSION 6 – INTERACTIONS WITH THE ENVIRONMENT**  
Chair: Andrea Eveland Talks 16-21. Pages 46-51.

- 8:00 AM **Li Guo, China Agricultural University** [T16]  
*Stepwise cis-regulatory changes in ZCN8 contribute to maize flowering time adaptation*
- 8:20 AM **Mon-Ray Shao, Donald Danforth Plant Science Center** [T17]  
*Quantifying maize root-shoot plasticity and 3D architectural changes from water stress using precision phenotyping*
- 8:40 AM **Alisa Huffaker, University of California – San Diego** [T18]  
*Genetic and biochemical delineation of the zealexin biosynthetic pathway reveals coordinated activity of multiple gene clusters to ensure production of a core maize defense*
- 9:00 AM **Davide Sosso, Inari Agriculture Inc.** [T19]  
*Improving maize NUE through multiplexed genome editing*
- 9:20 AM **Jiahn-Chou Guan, University of Florida** [T20]  
*Strigolactone deficient maize dramatically reduces parasitism by the “witchweed”, Striga, and reveals other unknown stimulants.*
- 9:40 AM **Stephanie Klein, Pennsylvania State University** [T21]  
*Root metaxylem as a novel target for improved drought tolerance in maize*

10:00 AM – 10:40 AM **BREAK**

10:40 AM – 12:30 PM **SESSION 7 – INVITED SPEAKERS**  
Chair: Andrea Gallavotti Pages 28 & 29.

- 10:40 AM Introduction
- 10:50 AM **Dominique Bergmann, Stanford University** [IS3]  
*Making a difference: stomatal pattern, form and function across plants*
- 11:40 AM **Erik Vollbrecht, Iowa State University** [IS4]  
*Shoot and inflorescence architecture in maize*





## **Sunday, March 17**

7:00 AM – 8:20 AM      **BREAKFAST** (Midway)

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

8:20 AM – 10:00 AM      **SESSION 9 – GENOME BIOLOGY AND EVOLUTION**  
Chair: Jeff Ross-Ibarra      Talks 28-32. Pages 58-62.

8:20 AM      **Bill Ricci, University of Georgia**      [T28]  
*Evidence of widespread gene-distal cis-regulatory elements in the maize genome*

8:40 AM      **Yong Peng, Huazhong Agricultural University**      [T29]  
*Three-dimensional chromatin interactions reveals the functional maize genome*

9:00 AM      **Kyle Swentowsky, University of Georgia**      [T30]  
*TR1 knobs become motile neocentromeres in the presence of a kinesin-14-like motor protein encoded on Ab10*

9:20 AM      **Benjamin Berube, Cold Spring Harbor Laboratory**      [T31]  
*Epigenetic perturbation of male meiosis in *Zea mays**

9:40 AM      **Patrick Monnahan, University of Minnesota**      [T32]  
*More references, more questions: Limitations in maize annotations that leads to different representations of gene models across maize reference genomes*

10:00 AM – 10:30 AM      **BREAK**

10:30 AM – 11:40 PM      **SESSION 10 – EXPRESSING THE GENOME**  
Chair: Todd Jones      Talks 33-35. Pages 63-65.

10:30 AM      **Robert Maple, University of Warwick**      [T33]  
*Meiosis-associated argonaute (MAGO) proteins are necessary for protecting the germline from misregulated transposable elements in maize.*

10:50 AM      **Hao Wu, Iowa State University**      [T34]  
*Investigation of gene regulatory network of maize endosperm development*

11:10 AM      **Maria Katherine Mejia Guerra, Cornell University**      [T35]  
*Decoding the transcriptional regulatory atlas of the maize leaf*

11:30 AM      **CLOSING REMARKS**

11:40 AM      **ADJOURNMENT**

# Posters

## Computational and Large-Scale Biology

- P1 **Lisa Harper**  
<[lisaharper@me.com](mailto:lisaharper@me.com)>  
*Next generation data management*
- P2 **Jack Gardiner**  
<[jack.m.gardiner@gmail.com](mailto:jack.m.gardiner@gmail.com)>  
*Using MaizeMine for genomic data integration and meta-analysis*
- P3 **John Portwood**  
<[john.portwood@ars.usda.gov](mailto:john.portwood@ars.usda.gov)>  
*MaizeGDB 2019: the maize multi-genome genetics and genomics database.*
- P4 **Christopher Topp**  
<[ctopp@danforthcenter.org](mailto:ctopp@danforthcenter.org)>  
*3D time lapse analysis reveals multiscale relationships in contrasting maize root architectures*
- P5 **Evan Rees**  
<[err87@cornell.edu](mailto:err87@cornell.edu)>  
*A fast computational pipeline for de novo assembly of plant genomes from long reads*
- P6 **Brandi Sigmon**  
<[bsigmon2@unl.edu](mailto:bsigmon2@unl.edu)>  
*A genome-wide association study of maize inflorescence traits and their plasticity under nitrogen stress*
- P7 **Paul Chomet**  
<[paul.chomet@nrgenome.com](mailto:paul.chomet@nrgenome.com)>  
*A novel solution to describe and manage maize genomic variation for high resolution genotyping*
- P8 **Arun Seetharam**  
<[arnstrm@iastate.edu](mailto:arnstrm@iastate.edu)>  
*A novel, evidence-weighted pipeline for improving maize gene structure annotations*
- P9 **Jason Wallace**  
<[jason.wallace@uga.edu](mailto:jason.wallace@uga.edu)>  
*A survey of the maize-associated microbiota in georgia*
- P10 **Toni Kazic**  
<[kazict@missouri.edu](mailto:kazict@missouri.edu)>  
*A system for managing maize research crops*
- P11 **Jaydon Lynch**  
<[lynchj21@montclair.edu](mailto:lynchj21@montclair.edu)>  
*A web server for Helitron identification*
- P12 **Zhenyuan Lu**  
<[luj@csihl.edu](mailto:luj@csihl.edu)>  
*Accessing MaizeCODE data via SciApps*
- P13 **Jerald Noble**  
<[jnoble333@ufl.edu](mailto:jnoble333@ufl.edu)>  
*Alternative splicing in the NAM lines using multiple reference genomes.*
- P14 **Thomas Hoban**  
<[thoban19@comcast.net](mailto:thoban19@comcast.net)>  
*Augmenting maize image training datasets using simulated photos*
- P15 **Erin Sparks**  
<[esparks@udel.edu](mailto:esparks@udel.edu)>  
*Bracing for Impact: The function of aerial roots in maize stability*
- P16 **Lauren Whitt**  
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- P272 **Jose Valdes Franco**  
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- P273 **Mike White**  
<[mrwhite4@wisc.edu](mailto:mrwhite4@wisc.edu)>  
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- P274 **Christopher Topp**  
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- P275 **Kelly Swarts**  
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- P276 **Stephanie Coffman**  
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- P277 **John Juvik**  
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*Breeding for natural food and beverage colorants from corn*
- P278 **Travis Rooney**  
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- P279 **Marlee Labroo**  
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- P280 **Armin C. Hoelker**  
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*Comparison of sampling strategies to maximize accuracy of genomic prediction in maize landraces*
- P281 **Stefan Hey**  
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- P282 **Cassandra Winn**  
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- P283 **Zhengbin Liu**  
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- P284 **Garrett Janzen**  
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- P285 **Nicholas Baert**  
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- P286 **Eric G. González-Segovia**  
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- P287 **Chenglong Wang**  
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- P288 **Margarita Mauro-Herrera**  
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- P289 **Aaron Kusmec**  
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- P290 **Adam Vanous**  
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- P293 **Brian Rice**  
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- P294 **Alain Charcosset**  
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- P295 **Qi Mu**  
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- P298 **Kevin Lehner**  
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*Field-based measurement of pulling force accelerates the identification of loci controlling root system architecture in maize*
- P299 **Tes Dennison**  
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- P301 **Guillaume Ramstein**  
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- P303 **Kyle King**  
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- P304 **Di Wu**  
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- P305 **Jinlong Li**  
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- P306 **Ruairidh Sawers**  
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- P307 **Kathryn Michel**  
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*Genome assembly of five Stiff Stalk inbreds: PHJ40, LH145, PHB47, NKH8431, & B84*
- P308 **Preston Hurst**  
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- P309 **Ricardo Andrade Pinto Jr**  
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*Genome wide association study for resistance to foliar diseases in corn lines*
- P310 **Barbara Muller**  
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*Genome-wide association analyses of maize kernel traits in the Wisconsin diversity (WiDiv) panel*
- P311 **Laura Tibbs**  
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*Genome-wide association studies of B vitamin levels in maize grain using 16.7 million imputed SNPs*
- P312 **Di Wu**  
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*Genome-wide association study of mineral levels in maize grain reveals candidate genes for mineral uptake and transport*
- P313 **Meng Lin**  
<[ml2498@cornell.edu](mailto:ml2498@cornell.edu)>  
*Genome-wide association study reveals the genetic architecture of leaf cuticular evaporation rate in maize*
- P314 **Mahule Elyse Boris Alladassi**  
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*Genotype imputation from Maize Association Panel to Ames Panel*
- P315 **Vinicius Costa Almeida**  
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*Hayman's diallel analysis for studying the inheritance of aluminum tolerance in tropical popcorn*
- P316 **Andrew Leakey**  
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*High-throughput phenotyping of maize root system size and distribution in the field through data extraction from minirhizotron images using machine learning*
- P317 **Lucas Roberts**  
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- P318 **Michael Busche**  
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*Identification of inbred-specific modifiers for narrow odd dwarf (nod)*
- P319 **Yuting Qiu**  
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*Identification of quantitative trait loci effective against bacterial leaf streak of maize*
- P320 **Zhongjie Ji**  
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*Identifying and parameterizing corn leaf and canopy characteristic for crop modeling*
- P321 **Yan Zhou**  
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- P322 **Sara Tirado**  
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*Image-based phenotypic platform for monitoring maize growth to estimate end-season productivity*
- P323 **Joseph Gage**  
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- P324 **Hongwei Zhang**  
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- P326 **Francielly Pereira**  
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- P328 **Zafar Wazir**  
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- P329 **Christopher Morales Farfan**  
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- P330 **Arthur Silva**  
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- P331 **Leo Zeitler**  
<[leo.zeitler@gmail.com](mailto:leo.zeitler@gmail.com)>  
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- P332 **Sergio Pérez-Limón**  
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*Mapping morphological traits characteristic of Mexican highland maize*
- P333 **Chen Chen**  
<[chenc71@163.com](mailto:chenc71@163.com)>  
*Mapping of maternal QTLs affecting in vivo haploid induction in maize (*Zea mays* L.)*
- P334 **Sarah Pedersen**  
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*Mapping the genetic architecture of maize adaptation to the Mexican highlands*
- P335 **Alejandro Ledesma**  
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*Molecular and phenotypic characterization of doubled haploids lines derived from different cycles of recurrent selection of the Iowa Stiff Stalk Synthetic (BSSS) maize population*
- P336 **Guri Johal**  
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*Natural diversity in *Slm1*, a highly inducible and autoactive maize immune receptor of the NLR class*
- P337 **Laura Manerus**  
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- P338 **Tingting Guo**  
<[tguo@iastate.edu](mailto:tguo@iastate.edu)>  
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- P339 **Jay Hollick**  
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*Parental RNA polymerase IV conditions heterotic traits*
- P340 **Andrew Leakey**  
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*Phenomics of stomata and water use efficiency in C4 species*
- P341 **Zubair Ahmed**  
<[zubairnarc15@gmail.com](mailto:zubairnarc15@gmail.com)>  
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- P343 **Diego Jarquin**  
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- P344 **Peter Balint-Kurti**  
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*Production of maize chromosome segment substitution line populations for the identification of loci associated with multiple disease resistance*
- P345 **Semra Palali Delen**  
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- P346 **Xi Wang**  
<[13297032291@163.com](mailto:13297032291@163.com)>  
*QTG-seq accelerates QTL fine mapping through QTL partitioning and whole-genome sequencing on bulked segregant samples*
- P347 **Jeffery Gustin**  
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*QTL analysis of maize seedling cold tolerance using Vigor: a machine vision assay for seedling emergence.*
- P348 **Leandro Neves**  
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*Repeat depletion for high-throughput sequencing of exonic and low copy regions of the maize genome*
- P349 **Adam Bray**  
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- P350 **Zihao Zheng**  
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*Shared genetic control of root system architecture between Zea mays and Sorghum bicolor*
- P351 **Leandro Zuffo**  
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- P352 **Christine O'Connor**  
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*Structural variation analysis in the Wisconsin Diversity Panel using short read sequence data and multiple de novo genome assemblies*
- P353 **Xuan Zhang**  
<[XuanZhang@cau.edu.cn](mailto:XuanZhang@cau.edu.cn)>  
*The genetic architecture of nodal root number in maize*
- P354 **Andrew Herr**  
<[awherr@iastate.edu](mailto:awherr@iastate.edu)>  
*Towards large scale corn root phenotyping: Designing and implementing genomic prediction*
- P355 **Jacob Washburn**  
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*Towards predictive maize breeding in the GxExM space*
- P356 **James McNellie**  
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*Understanding and predicting phenotypic plasticity with Joint Genomic Regression Analysis (JGRA)*
- P357 **Jonathan Renk**  
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*Understanding compositional changes during the alkaline cooking of maize (Zea mays L.) to mitigate acrylamide formation*
- P358 **Thomas Lubberstedt**  
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*Using spontaneous haploid genome doubling to access favorable alleles in exotic germplasm*
- P359 **Baoxing Song**  
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*Whole genome sequencing and de novo assembly for Andropogoneae species*
- P360 **Keith Duncan**  
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*X-ray imaging across scales in maize and sorghum biology: centimeters to micrometers*
- P361 **Yanyan Jiao**  
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*Xenia effect on oil content in maize embryo by hetero-fertilization*
- P362 **Yameng Liang**  
<[yml1992@cau.edu.cn](mailto:yml1992@cau.edu.cn)>  
*ZmMADS69 functions as a flowering activator through the ZmRap2.7-ZCN8 regulatory module and contributes to maize flowering time adaptation*

## **Transposons & Epigenetics**

- P363 **Charles Du**  
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*A sequence-indexed reverse genetics resource for maize: a set of lines with single Ds-GFP insertions spread throughout the genome*
- P364 **Meixia Zhao**  
<[MeixiaZhao@miamioh.edu](mailto:MeixiaZhao@miamioh.edu)>  
*An examination of the effects of epigenetic modifications and active transposable elements on meiotic recombination in maize*
- P365 **Jaelyn Noshay**  
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*Analysis of polymorphic TE insertions in maize reveals family specific influences on chromatin insertion site preference and spreading of DNA methylation*

- P366 **Jason Lynn**  
<[jlynn@bio.fsu.edu](mailto:jlynn@bio.fsu.edu)>  
*Characterization of b1 tandem repeat chromatin proteome in enhancement, silencing, and paramutation*
- P367 **Wei Guo**  
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*Dissecting the maintenance of MuDR transposon silencing in maize*
- P368 **Gen Xu**  
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*DNA methylation footprints during maize domestication and improvement*
- P369 **Cristian Forestan**  
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*Dynamic response to extended drought and recovery involve epigenetic control in stress adaptation and flowering regulation, providing insights into epigenetic memory in maize*
- P370 **Bosen Zhang**  
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*Dynamics of inheritance for small RNAs in maize breeding populations and their association with quantitative trait variation*
- P371 **Stefania Vendramin**  
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*Epigenetic regulation of ABA-induced transcriptional responses in maize*
- P372 **Clémentine Vitte**  
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*Genome-wide hypermethylation of TEs and centromeres following low temperature exposure in maize*
- P373 **Jonathan Gent**  
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*Heterochromatic 24nt siRNAs without RdDM in rice and maize*
- P374 **Maike Stam**  
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*Identification and characterization of regulatory sequences in Zea mays*
- P375 **Na Wang**  
<[na.wang25@uga.edu](mailto:na.wang25@uga.edu)>  
*Maize centromeres expand in the larger genome background of Oaxaca and Zea. luxurians*
- P376 **Thelma Madzima**  
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*MOP1-mediated transcriptional regulation of developmental genes under abiotic stress*
- P377 **Shujun Ou**  
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*The divergence of NAM founders revealed by syntenic LTR retrotransposons*
- P378 **Daniel Laspisa**  
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*The landscape of centromeres and pericentromeres of Zea mays*
- P379 **Benjamin Oakes**  
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*The roles of RNA polymerase IV and the environment in effecting heritable regulatory changes*
- P380 **Weijia Su**  
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*TIR-Learner, a new ensemble method for TIR Transposable Element annotation, provides evidence for the impact of TIR TEs on Maize Genome Structure and Evolution*
- P381 **Allison McClish**  
<[mcclish.23@osu.edu](mailto:mcclish.23@osu.edu)>  
*Transcriptional regulation of the maize genome*
- P382 **Sarah Anderson**  
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*Transposable element contributions to maize genome variation*
- P383 **Michelle Stitzer**  
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*Transposable elements contribute to adaptive response in selected maize populations*
- P384 **Sharu Paul Sharma**  
<[sharu@iastate.edu](mailto:sharu@iastate.edu)>  
*Transposon-induced inversions activate tissue specific gene expression in maize*
- P385 **Ryan Shontell**  
<[ryankhs@hawaii.edu](mailto:ryankhs@hawaii.edu)>  
*Understanding centromeric retrotransposons I – identification of protease cut sites and assembly of virus-like particles*
- P386 **Peter Crisp**  
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*Variation and inheritance of small RNAs in maize inbreds and their hybrids*

# **Invited Speaker Abstracts**

Invited Speaker 1

Friday, March 15 10:50 AM



## **Unveiling and harnessing mechanisms of epistasis and quantitative variation in plants**

(presented by Zachary Lippman <[lippman@cshl.edu](mailto:lippman@cshl.edu)>)

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Strong convictions often emerge when debating the significance of epistasis in plant evolution, domestication and breeding. I will share a remarkable multi-faceted case of epistasis in tomato that captures meristem development, flower production, gene family evolution, cryptic mutations, structural variation, dosage, selection, mechanized agriculture, and yield. What we have learned from the sum, or rather the interaction, of all of the above is guiding our current and future efforts in using genome editing to unveil and harness mechanisms of epistasis and quantitative variation for both basic biology and crop improvement.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), US-Israel Binational Agricultural Research and Development Fund (BARD)





## **The genetics and consequences of maize domestication and breeding**

(presented by Sherry Flint-Garcia <[sherry.flint-garcia@ars.usda.gov](mailto:sherry.flint-garcia@ars.usda.gov)>)

Full Author List: Flint-Garcia, Sherry<sup>1 2</sup>; Maize Diversity Project, .<sup>3</sup>; Maize ATLAS Project, .<sup>4</sup>; HiLo Highland Adaptation Project, .<sup>5</sup>

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The processes of domestication and breeding have had serious consequences on modern maize, particularly for the genes that are directly responsible for the transitions from the weedy teosinte ancestor to landrace populations to modern corn. Selection on these genes has so greatly reduced their genetic variation that no progress can be made by manipulating them in modern germplasm pools.

While there is an amazing amount of genetic and phenotypic diversity in landraces and teosinte, efficient utilization of this diversity for maize improvement is impeded by several phenomena: inbreeding depression, latitudinal adaptation (photoperiod response), and adaptation to elevation. In addition, the focus on yield as the primary trait of interest for the last several centuries has resulted in a loss of variation for food quality traits. My research program focuses on seed traits and seeks to integrate research on inbreeding and adaptation to develop better tasting and more nutritious food corn.

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



**Adjusting the valves: strategies for optimal stomatal development**(presented by Dominique Bergmann <[dbergmann@stanford.edu](mailto:dbergmann@stanford.edu)>)Full Author List: Bergmann, Dominique C<sup>1</sup><sup>1</sup> Stanford University and HHMI, Stanford, CA, USA, 94305-5020

Plant biologists are acutely aware of scale. Regardless of the level of our own studies, our research subjects confront us with the interconnectedness of their parts and processes. Fortunately, our plant research community is also interconnected, and this enables both broad and deep exploration of fundamental biological questions: at the smaller scales, we gain precision and rigor, moving up in scale, we gain impact. Scale is exceedingly important when considering stomata, the epidermal valves that regulate carbon dioxide and water vapor exchange between plants and the atmosphere. Stomatal guard cells and the developmental pathways used to make, pattern and adapt them to the prevailing environment offer a distillation of important themes in development, and a platform for single-cell investigations of identity and physiology. It is also an exciting time to forge links between stomatal development and physiology at leaf, plant, plot and ecosystem scales in diverse plant families. In addition, the parallel expansion of stomatal lineage complexity and stomatal transcription factors across the plant kingdom provides a powerful “natural laboratory” in which to analyze structure/function of individual proteins and evolution of their regulatory networks. In my talk, I will present vignettes of how we’ve leveraged key regulators of stomatal development into a system-wide view of cell identity, and preview some of the experimental and imaging-based tools that enable us to capture and manipulate these regulators of developmental decisions in *Arabidopsis*. With these tools and the discovery that homologous transcription factors anchor cell identities across the plant kingdom, we are beginning to capture information about cell identity and developmental potential in a wide range of plants possessing interesting stomatal properties, including many plants of ecological and economic relevance.

Funding acknowledgement: Howard Hughes Medical Institute (HHMI)



## Shoot and inflorescence architecture in maize

(presented by Erik Vollbrecht <[vollbrec@iastate.edu](mailto:vollbrec@iastate.edu)>)

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Architecture and construction of a mature plant shoot is a readout of developmental processes that were executed through ontogeny. In corn belt maize, a single vegetative shoot predominates and there are two types of reproductive shoots or inflorescences, the tassel and ear. Shoots consist of axes (*i.e.*, stems and branches) and their lateral organs (*e.g.*, leaves or floral organs), components that all initiate and develop from shoot apical meristems. Thus, the architecture of a particular shoot reflects the number, arrangement and activity of shoot meristems, and the differential elaboration of meristem products during development. Each inflorescence develops from hundreds of meristems and a handful of meristem types and genetic approaches have identified mutants that regulate those meristem activities. Among them, the *ramosa* genes participate in a long branch/short branch developmental decision that underlies the basic architectural difference between tassels and ears, and help define a core genetic network for inflorescence branching. Using a variety of forward and reverse genetic approaches including QTL and mutagenesis screens for loci that modify the phenotype of weak *ramosa* mutant alleles, we have tested several hypotheses suggested by that emerging gene regulatory network. These studies have identified transcription factor and plant growth regulator modules that interact in mostly additive fashion to fine-tune branching in the inflorescences, with surprisingly little evidence for the kind of epistasis observed when combining *ramosa* mutants. In the maize vegetative shoot, apical dominance during development results in a single major axis and elaboration of leaves, including the important agronomic trait of leaf angle, is a major component of shoot architecture. In both the vegetative and inflorescence shoots, the activities of meristems are regulated non-cell autonomously by genes expressed adjacent to the meristems, and genes identified from mutant studies show evidence of selection during domestication. Together, these genetic determinants suggest strategies to affect yield by regulating the developmental basis of a range of shoot and inflorescence architecture traits.

Funding acknowledgement: National Science Foundation (NSF)

# McClintock Prize Abstract

McClintock Prize

Friday, March 15 8:10 PM



## **Epistasis, the spice of life: Lessons from the study of the plant immune system**

(presented by: Detlef Weigel <[detlef.weigel@tuebingen.mpg.de](mailto:detlef.weigel@tuebingen.mpg.de)>)

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My group is addressing fundamental questions in evolutionary biology, using both genome-first and phenotype-first approaches. A few years ago, we discovered that *Arabidopsis thaliana* is a great model for the study of hybrid necrosis. This widespread syndrome of hybrid failure in plants is caused by plant paranoia – regardless of the presence of enemies, plants “think” they are being attacked by pathogens. The consequence is autoimmunity, which can be extreme enough to kill plants before they set seeds.

Over the past decade, we have studied in detail the underlying genetics, finding that often only one or two loci are involved, with most of them encoding NLR immune receptors. The NLR gene family is the most variable gene family in plants, and it is thus not surprising that they are often involved in genome-genome conflict, with alleles at one locus greatly changing the activity of alleles at another locus. Similarly, we have found that autoimmunity due to allelic variation at the ACD6 locus, which probably encodes a channel, is modulated by a slew of extragenic suppressors. I will describe what we have learned and how our unique angle on studying the plant immune system has led to insights that were not obtained with conventional laboratory genetics.

Our goal for the next decade is to understand the genomic and geographic patterns of immune system diversity. Together with collaborators Jeff Dangl, Jonathan Jones and Brian Staskawicz, we have been describing species-wide diversity of NLR immune receptor genes. In parallel, we have been describing with collaborator Eric Kemen the local diversity on *A. thaliana* plants of the microbial pathogen, *Pseudomonas*, on *A. thaliana* plants. This year, we initiated an ambitious new project, Pathodopsis (Patho[gen]s in Arabi[dopsis]), in which we aim to describe genetic diversity in the host and two important pathogens, the generalist *Pseudomonas* and the specialist *Hyaloperonospora arabidopsidis*. The long-term vision is to produce maps of resistance alleles in the host, and of effector alleles in the pathogens, in order to learn who normally wins in a wild plant pathosystem – the host or the pathogen.

Additional information about our work can be found on our websites, <http://weigelworld.org> and <http://pathodopsis.org>.

## **Short Talk Abstracts**

**SESSION 1 – COMMUNICATING WITHIN AND BETWEEN CELLS AND PLANTS**

Chair: Hilde Nelissen

Thursday, March 14. 7:00 PM – 9:00 PM



T1

### **Role of the microtubule-binding protein TANGLED1 in plant cell division and growth**

(presented by Carolyn Rasmussen <[crasmu@ucr.edu](mailto:crasmu@ucr.edu)>)

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How cells within multicellular organisms coordinately grow and divide are fundamental questions in plant biology. Pattern formation within tissues and organs relies on proper integration of cell-cell communication and developmentally regulated growth and division. We study how division plane orientation promotes proper growth in maize by analysis of a mutant, *tangled1*, that has defects in division plane orientation and delayed mitotic progression. Our recent in vitro data suggest that TANGLED1 (TAN1) protein binds microtubules with high affinity. Further, real-time microtubule dynamic assays indicates that TAN1 promotes microtubule crosslinking. To investigate how TAN1-microtubule crosslinking affects cell shape and division patterns in vivo, we use a cell-shape based mathematical model to predict division planes that divide the cell into two equal daughter cells while minimizing new cell wall surface area. Preliminary evidence suggests that irregular cell shape in *tan1* mutant affects the ability of *tan1* mutant cells to properly place divisions, suggesting that aberrant cell shape itself may feed forward to future improper division plane placement. To investigate whether TAN1-microtubule crosslinking is essential for either interphase or mitotic microtubule organization, we are analyzing microtubule dynamics in the *tan1* mutant. These studies will provide insight into how microtubule organization contributes to division plane orientation and growth in maize and other plants.

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



T2

## ***Tasselseed5* overexpresses a wound-inducible enzyme, *ZmCYP94B1* that affects jasmonate catabolism, sex-determination, and plant architecture in maize**

(presented by China Lunde <[lundec@berkeley.edu](mailto:lundec@berkeley.edu)>)

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Maize is monoecious, with separate male and female inflorescences. Maize flowers are initially bisexual but achieve separate sexual identities through organ arrest. Loss-of-function mutants in the jasmonic acid (JA) pathway have only female flowers due to failure to abort silks in the tassel. *Tasselseed5* (*Ts5*) shares this phenotype but is dominant. Positional cloning and transcriptomics of tassels identified an ectopically expressed gene in the CYP94B subfamily as *Ts5* (*ZmCYP94B1*). CYP94B enzymes are wound-inducible and inactivate bioactive jasmonoyl-L-isooleucine (JA-Ile). Consistent with this result, tassels and wounded leaves of *Ts5* mutants displayed lower JA and JA-Ile precursors and higher 12OH-JA-Ile product than wild type. Furthermore, many wounding and jasmonate pathway genes were differentially expressed in *Ts5* tassels and overexpression of *ZmCYP94B1* in Arabidopsis phenocopies the *cyp94b3* loss-of-function phenotype. We propose that the *Ts5* phenotype results from interruption of JA signaling during sexual differentiation via upregulation of *ZmCYP94B1* and that its proper expression maintains maize monoecy.

Gene / Gene Models described: *Ts5*; GRMZM2G177668

Funding acknowledgement: National Science Foundation (NSF)



T3

## **Maize multi-omics reveal roles for autophagic recycling in amino acid, nucleotide and carbohydrate metabolism during carbon starvation.**

(presented by Fionn McLoughlin <[fmcloughlin@wustl.edu](mailto:fmcloughlin@wustl.edu)>)

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Autophagy plays an important role in plant nutrient recycling by removing unwanted or dysfunctional membranes, protein complexes and organelles. This cellular material is sequestered into double membrane-bound autophagosomes, which are transported and released into the vacuole for breakdown and recycling. Consequently, plants defective in autophagy accumulate aberrant proteins/organelles, and are sensitive to reduced nutrient availability. Central to this process is the AUTOPHAGY-RELATED (ATG)-8 protein, which coats the enveloping autophagosome after its conjugation to the lipid phosphatidylethanolamine (PE). The Atg8-PE adduct then provides a docking platform for adaptors that drive vesicle closure and vacuolar fusion, and receptors that select appropriate cargo. ATG12 is an important mediator of ATG8 lipidation and, consequently, atg12 mutants fail to assemble Atg8-PE adducts and effectively lack Atg8-mediated autophagic recycling. To better define the cellular processes impacted by autophagy and its importance for crop yields, we combined proteomic, transcriptomic, metabolomic and ionomic approaches to pinpoint those that are affected in response to limited carbon availability. Collectively, the omics profiles of WT and atg12 leaves before and after local shading revealed central roles for autophagy in regulating carbohydrate complexity and content, with a decrease in several complex carbohydrates such as maltose and maltotetraose being observed in atg12 leaves, while simple carbohydrates like fructose-6-phosphate and rhamnose were increased up to 16-fold. In addition, a striking increase in various free amino acids was seen, together with the accumulation of purine and pyrimidine degradation products, indicating that autophagy is required for the correct processing of these metabolites, or that they are retained to maintain normal carbon:nitrogen ratios. Together, these studies provide insight into processes impacted by autophagy, which should facilitate the development of crops that are more tolerant to nutrient deprivation and have improved nutritional value.

Funding acknowledgement: National Science Foundation (NSF)

T4

## **Pollen vegetative cell and sperm cell transcriptomes help predict mutation effects on fertilization success**

(presented by Matthew Warman <[warmanma@oregonstate.edu](mailto:warmanma@oregonstate.edu)>)

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In flowering plants, the haploid male gametophyte (pollen grain and pollen tube) is essential for sperm delivery, double fertilization, and subsequent initiation of seed development. Despite this central role in reproduction, mechanisms enabling rapid pollen tube growth and fertilization remain relatively unknown. To help identify components of these processes, we quantified transcript levels in four male reproductive stages (tassel primordia, microspores, mature pollen, and sperm cells) via RNA-seq. To test the hypothesis that high expression correlates with functional relevance, we measured the male-specific fitness cost (i.e., transmission deficit in a heterozygous outcross) of GFP-tagged exon insertion alleles from the Dooner/Du collection (Li et al., 2013) in over 50 genes highly expressed (top 20% by FPKM) in mature pollen, sperm cells, or seedling (as a sporophytic control). A new imaging platform and software pipeline allowed efficient categorization of phenotypes for >150,000 seeds, enabling sensitive detection of non-Mendelian segregation. Insertions in genes highly expressed only in seedling or primarily in sperm cells (with one exception) exhibited no difference from the expected ratio. In contrast, insertions in nearly 40% of the pollen vegetative cell genes tested had small but significant effects on fitness ( $\chi^2$ ,  $p < 0.05$ ), with the frequency increasing to three-quarters in the subset of the most highly expressed genes (top 5% by FPKM). Intriguingly, the single defect linked to a sperm cell gene was strong (28% transmission) and associated with partial seed filling and embryo/endosperm non-concordance when crossed as a male, suggesting a role at fertilization. These data support the idea that a large set of genes expressed in the vegetative cell, some with small effects, contributes to competitive pollen tube growth, making it broadly sensitive to genetic perturbation. We estimate that mutations in more than 1200 pollen vegetative cell genes could result in non-Mendelian segregation.

Funding acknowledgement: National Science Foundation (NSF)



T5

## An “a-maizing” connection between cell wall biosynthesis and carbohydrate partitioning: *Brittle Stalk 2-Like3* encodes *carbohydrate partitioning defective28*

(presented by Benjamin Julius <[btjg2d@mail.missouri.edu](mailto:btjg2d@mail.missouri.edu)>)

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Carbohydrate partitioning, the process of transporting carbohydrates from photosynthetic source tissues, where the sugars are synthesized, to non-photosynthetic sink tissues, is required by plants for proper growth and development. This complex process has been well studied from physiological, anatomical, and biochemical perspectives; however, many questions concerning the genetic control of carbohydrate partitioning remain unanswered. The recessive *carbohydrate partitioning defective28* (*cpd28*) and *carbohydrate partitioning defective47* (*cpd47*) mutants were identified from an EMS mutagenized population based on phenotypes associated with increased carbohydrate accumulation, such as leaf chlorosis, decreased plant vigor, and accumulation of starch and soluble sugars in the source leaves. The two mutant lines were determined to be allelic following genetic complementation tests. Transport studies with C14-sucrose found drastically decreased export from mature leaves in *cpd28* and *cpd47* relative to wild-type siblings, resulting in a 4-15x increase in soluble sugars and starch in the mutant leaves. Using a whole genome sequencing approach, we identified the causative mutation for each allele in the *Brittle Stalk 2-Like3* (*BK2L3*) gene, a member of the COBRA family involved in cell wall development across monocots and dicots. Interestingly, none of the previously characterized COBRA genes are reported to affect carbohydrate partitioning. In agreement with other characterized COBRA members, the BK2L3 protein localizes to the plasma membrane, and both mutant alleles condition a dwarf phenotype in dark-grown shoots and primary roots. Likewise, both alleles exhibit a significant crystalline cellulose deficiency in mature source leaves. Therefore, *Brittle Stalk 2-like3* functions in tissue growth, potentially through tissue-specific cell wall development, and unexpectedly impacts carbohydrate partitioning. This work demonstrates an unprecedented connection between cellulose biosynthesis and whole-plant carbohydrate partitioning.

Gene / Gene Models described: *bk2l3*; Zm00001d034049

Funding acknowledgement: National Science Foundation (NSF)



T6

**TeoNAM: A nested association mapping population for domestication and agronomic trait analysis**

(presented by Qiuyue Chen <[qchen295@wisc.edu](mailto:qchen295@wisc.edu)>)

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Recombinant inbred lines (RIL) are an important resource for mapping complex traits in many species. While several RIL populations have been developed for maize, a RIL population with multiple teosinte inbred lines has been lacking. Here, we report a teosinte nested association mapping population (TeoNAM), derived from crossing five teosinte inbreds to the maize inbred line W22. The resulting 1257 BC<sub>1</sub>S<sub>4</sub> RILs were genotyped with 51,544 SNP markers, which provides a high-density genetic map with a length of 1540 cM. On average, each RIL is 15% homozygous teosinte and 8% heterozygous. We performed joint linkage mapping (JLM) and genome-wide association analysis for 22 domestication and agronomic traits. A total of 255 QTLs from JLM were identified with many mapping to known genes and some to novel candidate genes. TeoNAM is an useful resource for QTL mapping with high resolution and discovery of novel allelic variation from teosinte.

Funding acknowledgement: National Science Foundation (NSF)



T7

## **Combining ability, per se yield components, and GxE in the Stiff Stalk heterotic group dissected using new genome assemblies combined with exome-capture genotyping of a multi-parent population**

(presented by Kathryn Michel <[kathryn.michel@wisc.edu](mailto:kathryn.michel@wisc.edu)>)

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<sup>7</sup> NA

The Stiff Stalk (SS) heterotic pool is a cornerstone of commercial U.S. cornbelt dent hybrids. Key founder inbreds with desirable per se characteristics for seed production and combining ability for hybrid production formed the basis of the current elite breeding pool. Six founder inbreds, or highly related derivatives – B73, B84, PHB47 (B37 type), LH145 (B14 type), PHJ40 (novel early SS), and NKH8431 (B73/B14 type)- were chosen to be parents of a multi-parent advanced generation intercross (MAGIC) population. We completed PacBio-based assemblies of the five non-B73 inbreds and compared them to the B73 reference. Multiple chromosomal aberrations, including inversions and presence-absence variants, were detected. The MAGIC population, consisting of 726 individuals, was evaluated per se during 2016 and 2017 for anatomical and grain yield component traits. Significant QTL peaks were found using markers derived from exome capture sequencing exemplified by QTL for kernel row number on chromosomes 3 and 5, and for growing degree units to anthesis on chromosomes 3 and 8. To assess yield and yield stability of inbreds from this heterotic pool, hybrids of an array of SS lines crossed to DK3IHH6 were evaluated in 31 environments as a part of the Genomes To Fields initiative. Consistent with expected outcomes of breeding for wide adaptation, inbreds randomly derived from BSSS C0 and first generation inbreds were less stable across environments than more recent inbreds. Hybrids from MAGIC lines were less stable on average than hybrids of the original six parents, but were more stable on average than BSSS C0 random-line hybrids. This observation supports heritability of stability, and demonstrates that lines with low stability can segregate from highly stable parents. The combination of advanced genome resources with novel mapping populations will continue to allow us to unravel the genetic architecture that supports valuable seed parent characteristics, combining ability/heterosis, and environmental stability that are hallmarks of this heterotic group.

Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE), Iowa Corn Promotion Board, Wisconsin Corn Promotion Board, National Corn Growers Association

T8

## **Genome-Phenome Wide Association Study (GPWAS): Using high dimensional phenotype data to identify the genes that specify the traits of maize**

(presented by Zhikai Liang <[zliang@huskers.unl.edu](mailto:zliang@huskers.unl.edu)>)

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Diverse computational biological tools were developed to provide evidences on supporting *in silico* annotated genes, however, only a small percentage of genes possess validated functional roles. GWAS attempts to link phenotypic variation and genetic variants across the genome. Currently, the number of plant phenotypes which have been characterized in the same association populations, and the number of environments in which those populations have been grown and phenotyped is rapidly growing. High throughput phenotyping technology will accelerate this trend, as it will become possible to measure many more distinct plant traits from the same set of raw plant sensor data. The expanded phenotypic datasets enable another method to test associations between specific genes and variation in target traits. Here we propose the Genome-Phenome Wide Association Study (GPWAS) model, which operates on the principle of reversing the relationship in the variables of a conventional GWAS. GPWAS simultaneously selects an optimum combination of phenotypes to explain maximum variants of a single gene in a population level and uses permutation testing to evaluate the statistical significance of this association. Using whole genome resequencing data from Maize HapMap3 with a set of simulated phenotypes, we demonstrate that GPWAS model can provide additional power relative to FDR when analyzing large sets of phenotypic data include many traits with low heritability. Using genotype and phenotype data of maize retrieved from Panzea, GPWAS detected genes were found to enrich many more GO terms and with greater statistical significance than genes detected by conventional GWAS models. Genes displayed by GPWAS tend are more likely to be independently validated than conventional GWAS models using the same dataset. In summary, these results suggest that genes identified by GPWAS are more likely to have plant functionally constrained roles in determining phenotype.



T9

## **Functional genetic diversity in the commercial germplasm pool – Is there anything left?**

(presented by Elizabeth Lee <[lizlee4@gmail.com](mailto:lizlee4@gmail.com)>)

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Additive genetic variation is key for plant breeders to continue to make genetic progress. The Commercial maize germplasm pool traces back to 7 key founder lines; it is highly stratified into heterotic patterns: Stiff Stalks one side of the hybrid pedigree and non-Stiff Stalks (Iodents, and non-Iodents) on the other side. And the commercial germplasm pool has generally remained closed to influxes of new genetic diversity. As part of the Genomes to Fields Initiative, we examined the consequences that over 70 years of genetic improvement has had on the Commercial germplasm pool. Using important off-PVP inbred lines and second-generation lines derived from the off-PVP lines, 3 subsets of lines were identified based on maturity: early maturity set, intermediate maturity set, and late maturity set. Each set consisted of 5-6 Stiff Stalk inbred lines and 16-17 non-Stiff Stalk inbred lines to generate approximately 96 hybrids. While the set of lines used are 4-5 breeding cycles removed from current germplasm, 40% of the Stiff Stalk lines and 65% of the non-Stiff Stalk lines used in this study are still represented in commercial hybrids. Furthermore, GBS data suggest that the set of lines captures the breadth of diversity of the commercial germplasm pool and a greater breadth of diversity than the NAM parents from cornbelt dent origins. Comprehensive yield trial results show that the commercial germplasm pool still has additive genetic variation present for grain yield, but that it is only present in the non-Stiff Stalk portion of the germplasm pool. Only environmental specific additive genetic variation was observed for grain yield in the Stiff Stalk germplasm pool. The lack of useful, functional genetic diversity in the Stiff Stalk germplasm pool has severe implications for continued genetic progress.

Funding acknowledgement: United States Department of Agriculture (USDA), Genomes to Fields, OMAFRA, NSERC

T10



## **Sequence, assembly and annotation of Bayer Crop Science's maize inbred line LH244; A new resource for maize genetics and transformation**

(presented by Ruth Wagner <[ruth.wagner@bayer.com](mailto:ruth.wagner@bayer.com)>)

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Access to elite, transformable germplasm is required to design and maintain transformation pipelines. Product pipelines typically use transformable germplasm to initially introduce the novel variant, which is then crossed into broad, diverse germplasm lines relevant to the geographies where the product will be grown. Effective transformation pipelines are valuable for product development in the Ag industry but are also important for serving the scientific community by enabling basic science research through gene and pathway discovery and characterization. Bayer Crop Science, in collaboration with NRGene and the University of Wisconsin, reports the release of the LH244 inbred maize transformation line germplasm and assembled reference genome to academic research communities. The germplasm will be released to public seed stock centers and the assembled, annotated genome and a physiological description of the line will be published, and resources for efficient transformation will be available to the University of Wisconsin Crop Innovation Center. LH244 is a commercially relevant inbred line that is readily transformable, thus making it a complete resource for genomic and genetic exploration. In this talk, we will share insights into the unique features of the LH244 genome, transformability and physiology that make it a foundation resource for the maize genetics community.

T11



## Next generation data management

(presented by Lisa Harper <[lisa.harper@ars.usda.gov](mailto:lisa.harper@ars.usda.gov)>)

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We are nearing at a time in history where we will have the potential to capture and curate almost all published maize data. Imagine if every piece of published biological data is available from easy-to-find and well-organized resources; the data are accurately described and available in accessible and standard formats; the experimental procedures, samples and time points are all completely documented; and researchers can find answers to any question about the data with just a few mouse clicks. This could affect all of our research in ways we cannot even yet imagine. However, the amount of published data has risen exponentially while curator person-hours have remained constant. To facilitate “next generation curation”, we only need to make a few simple changes to our workflows to have enormous positive effects in data viability. In this talk, we will outline how to achieve this, and ways that MaizeGDB can help.

Funding acknowledgement: United States Department of Agriculture (USDA)

T12

**The regulatory landscape of a core maize domestication module controlling bud dormancy and growth repression**(presented by Zhaobin Dong <[dongz@berkeley.edu](mailto:dongz@berkeley.edu)>)Full Author List: Dong, Zhaobin<sup>1</sup>; Xiao, Yuguo<sup>2</sup>; Govindarajulu, Raj<sup>3</sup>; Feil, Regina<sup>4</sup>; Lunn, John E.<sup>4</sup>; Hawkins, Jennifer S.<sup>3</sup>; Whipple, Clinton<sup>2</sup>; Chuck, George<sup>1</sup><sup>1</sup> Plant Gene Expression Center, UC Berkeley, Albany, CA, 94710, USA<sup>2</sup> Department of Biology, Brigham Young University, Provo, UT, 84602, USA<sup>3</sup> Department of Biology, West Virginia University, Morgantown, WV, 26506, USA<sup>4</sup> Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, 14476, Germany

Many domesticated crop plants have been bred for increased apical dominance, displaying greatly reduced axillary branching compared to their wild ancestors. In maize this has been achieved through selection for a gain of function allele of the TCP transcription factor *teosinte branched1* (*tb1*). Despite its importance, the precise genetic mechanism for how a dominant *Tb1* allele was able to achieve axillary bud suppression during domestication is unknown. By raising an antibody to TB1 and performing ChIP-seq on 1mm young axillary bud tissue coupled with RNA-seq, we identified the genetic pathways regulated by TB1. In addition we measured the levels of several hormones and sugars in young bud tissue from wildtype and *tb1* mutants, allowing us to correlate TB1 target gene activity to metabolic end products. Although the control of apical dominance has been well established to be mediated by auxin, measurements of auxin levels in wildtype and *tb1* buds showed no difference, and auxin-related genes were not the main category of differentially expressed genes or ChIP targets. Instead, several other hormone genes were identified as TB1 direct targets, including gibberellins, abscisic acid and jasmonic acid. Levels of the latter two were greatly reduced in *tb1* buds, as were several sugars, including those involved in signaling such as trehalose-6-phosphate. This demonstrates that TB1 achieves bud suppression through the production of inhibitory phytohormones as well as through the control of sugar levels and overall energy balance. Interestingly, TB1 also targets several other previously described domestication loci, including *teosinte glume architecture1* (*tga1*), the prolificacy QTL *prol1.1* upstream of *grassy tillers1* (*gt1*), as well as *tb1* itself. This fact places *tb1* near the top of the domestication hierarchy, demonstrating the critical importance of this gene during the domestication of maize from teosinte.

Funding acknowledgement: National Science Foundation (NSF)



T13



## The ZmbZIP22 transcription factor regulates 27-kD $\gamma$ -zein gene transcription during maize endosperm development

(presented by Li Chaobin <[chaobinli@126.com](mailto:chaobinli@126.com)>)

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Zeins are the most abundant storage proteins in maize (*Zea mays*) kernels, thereby affecting the nutritional quality and texture of this crop. 27-kD  $\gamma$ -zein is highly expressed and plays a crucial role in protein body formation. Several transcription factors (TFs) (O2, PBF1, OHP1, and OHP2) regulate the expression of the 27-kD  $\gamma$ -zein gene, but the complexity of its transcriptional regulation is not fully understood. Here, using probe affinity purification and mass spectrometry analysis, we identified ZmbZIP22, a TF that binds to the 27-kD  $\gamma$ -zein promoter. ZmbZIP22 is a bZIP-type TF that is specifically expressed in endosperm. ZmbZIP22 bound directly to the ACAGCTCA box in the 27-kD  $\gamma$ -zein promoter and activated its expression in wild tobacco (*Nicotiana benthamiana*) cells. 27-kD  $\gamma$ -zein gene expression was significantly reduced in CRISPR/Cas9-generated *zmbzip22* mutants. CHIP-seq (chromatin immunoprecipitation coupled to high-throughput sequencing) confirmed that ZmbZIP22 binds to the 27-kD  $\gamma$ -zein promoter in vivo and identified additional direct targets of ZmbZIP22. ZmbZIP22 can interact with PBF1, OHP1, and OHP2, but not O2. Transactivation assays using various combinations of these TFs revealed multiple interaction modes for the transcriptional activity of the 27-kD  $\gamma$ -zein promoter. Therefore, ZmbZIP22 regulates 27-kD  $\gamma$ -zein gene expression together with other known TFs.

Gene / Gene Models described: *bzip22*; GRMZM2G043600

Funding acknowledgement: National Natural Science Foundation of China (NSFC), National Key Research and Development Program of China, National Postdoctoral Program for Innovative Talents

T14

## Formation of the maize blade-sheath boundary: evidence for a prepattern (presented by Josh Strable <[jjs369@cornell.edu](mailto:jjs369@cornell.edu)>)

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Understanding gene networks that pattern boundaries in leaves is a central topic in plant biology with important applications in agriculture. The maize leaf consists of a stem-grasping proximal sheath and a distal blade hinged by auricle and ligule tissues that contribute to leaf angle. Proximal-distal identities are evident in plastochron 6 (P6) primordia, the sixth youngest leaf primordium in the seedling shoot; however, this boundary is morphologically inconspicuous in P4 primordia. We used laser microdissection RNA-seq (lmRNA-seq) to isolate four contiguous proximal-distal microdomains in P4 primordia that we examined for signals of boundary-like expression patterns. Our study identified 1,045 differentially expressed genes across these microdomains; self-organizing map clustering and gene ontology analyses indicate some nodes are significantly enriched for transcription factor activity and hormone regulation ontologies. This included microdomain-specific expression of *knotted1-like homeobox* genes, which are known to play key roles in regulating development of the blade-sheath boundary, and other organ boundary genes. These data suggest that leaf boundaries may be prepatterned by P4. We additionally uncovered microdomain-related expression of proximal cytokinin and distal auxin signaling genes. We found significant enrichment of previously uncharacterized *zinc-finger homeodomain (zhd)* genes in proximal microdomains and in the pre-ligule (~P6). Expression, genetic interaction and gene regulatory network inference analyses support a working model wherein proximally-expressed *zhd* genes redundantly target other genes controlling boundary development. We combined additional lmRNA-seq datasets from older leaf primordia and from *liguleless (lg)* mutants to conduct a weighted gene co-expression network analysis on 61 lmRNA-seq libraries and found *lg1* and *lg2* form a co-expression module as do *zhd* genes. This study underscores the complexity of gene and hormone networks that prepattern boundaries during leaf development.

Funding acknowledgement: National Science Foundation (NSF)

T15



## ***Few branched1* is a positional regulator of inflorescence architecture in maize**

(presented by Clinton Whipple <[whipple@byu.edu](mailto:whipple@byu.edu)>)

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Regulation of meristem determinacy is key to establishing inflorescence architecture, and important component of yield. However, the mechanism by which branching is positionally regulated to give distinct inflorescence architectures is poorly understood. As is typical of other species in the Andropogoneae, the maize inflorescence produces two distinct kinds of branches: indeterminate long branches at the base of the tassel, and determinate short branches (spikelet pairs) along the central spike. *Few branched1* (*Fbr1*) is a semi-dominant mutant of maize that affects only the long branch zone of the tassel inflorescence. In *Fbr1* mutants, long branches are frequently absent, leaving an empty node occasionally subtended by a bract that in some genetic backgrounds has a mosaic leaf/carpel or stamen identity. Positional cloning of *Fbr1-R* and confirmation by a second allele and transgenic phenocopy show that a single amino acid substitution in a non-conserved domain of a WUSCHEL-like homeobox (WOX) protein causes the gain-of-function phenotype. *Fbr1* transcripts are expressed in a boundary domain adjacent to branch meristems, suggesting that *Fbr1* acts non-cell autonomously. Interestingly the causative mutation is localized to a region of the protein known to regulate cell-cell movement through homodimerization. RNA-seq profiling demonstrates that multiple MADS-box floral regulators are ectopically expressed in *Fbr1-R* mutant tassel primordia, suggesting that *Fbr1* promotes branch meristem determinacy by promoting an ectopic and premature floral identity program. A model is emerging in which positional regulation of FBR1 protein homodimerization, and cell non-autonomous signaling promotes meristem determinacy of short branches in maize.

Funding acknowledgement: National Science Foundation (NSF)



T16

## Stepwise *cis*-regulatory changes in *ZCN8* contribute to maize flowering time adaptation

(presented by Li Guo <[guoli1992@cau.edu.cn](mailto:guoli1992@cau.edu.cn)>)

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Maize (*Zea mays* ssp. *mays*) was domesticated in southwestern Mexico ~9,000 years ago from its wild ancestor, teosinte (*Zea mays* ssp. *parviglumis*). From its centre of origin, maize experienced a rapid range expansion and spread over 90° of latitude in the Americas which required a novel flowering time adaptation. *ZEA CENTRORADIALIS 8* (*ZCN8*) is the maize florigen gene and has a central role in mediating flowering. Here, we show that *ZCN8* underlies a major quantitative trait locus (*qDTA8*) for flowering time that was consistently detected in multiple maize-teosinte experimental populations. Through association analysis in a large diverse panel of maize inbred lines, we identified a single nucleotide polymorphism (SNP-1245) in the *ZCN8* promoter that showed the strongest association with flowering time. SNP-1245 co-segregated with *qDTA8* in maize-teosinte mapping populations. We demonstrate that SNP-1245 is associated with differential binding by the flowering activator *ZmMADS1*. SNP-1245 was a target of selection during early domestication which drove the pre-existing early-flowering allele to near fixation in maize. Interestingly, we detected an independent association block upstream of SNP-1245, wherein the early-flowering allele that most likely originated from *Zea mays* ssp. *mexicana* introgressed into the early-flowering haplotype of SNP-1245 and contributed to maize adaptation to northern high latitudes. Our study demonstrates how independent *cis*-regulatory variants at a gene can be selected at different evolutionary times for local adaptation, highlighting how complex *cis*-regulatory control mechanisms evolve. Finally, we propose a polygenic map for the pre-Columbian spread of maize throughout the Americas.

Gene / Gene Models described: *ZCN8*; GRMZM2G179264

Funding acknowledgement: National Science Foundation (NSF), National Key Research and Development Program of China, National Natural Science Foundation of China, Recruitment Program of Global Experts

T17

## **Quantifying maize root-shoot plasticity and 3D architectural changes from water stress using precision phenotyping**

(presented by Mon-Ray Shao <[mshao@danforthcenter.org](mailto:mshao@danforthcenter.org)>)

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Limited water availability is one of the major threats to grain yields in agriculture. Although phenotypic responses to drought have long been of interest, whole plant-level changes have been difficult to quantify at large scale because of technical challenges, particularly regarding root architecture. We leveraged X-ray computed tomography to perform 3D phenotyping of post-anthesis root crowns excavated from the field for a subset of the Genomes to Fields maize germplasm. Significant architectural differences resulted from genotype and irrigation regimes, such as in average root radius and solidity. We found a strong positive correlation between root pulling force (RPF), a high-throughput field-based method for quantifying root systems, to X-ray based traits including 3D root volume, surface area, and total length, suggesting that valuable information about root systems can be obtained at large scale. In a seedling-based study, we grew the maize Nested Association Mapping founders in a LemnaTec time-course trial to investigate variation in root-shoot plasticity and root architecture under increasingly severe water limitation stress, identifying genotypes demonstrating the most plasticity versus stability, and traits associated with mature biomass. Together, our work provides a multi-faceted approach towards understanding variation in root and shoot growth dynamics and plant architecture under drought.

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

T18

## **Genetic and biochemical delineation of the zealexin biosynthetic pathway reveals coordinated activity of multiple gene clusters to ensure production of a core maize defense**

(presented by Alisa Huffaker <[ahuffaker@ucsd.edu](mailto:ahuffaker@ucsd.edu)>)

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Specialized metabolites are integral to maize anti-pathogen defenses. While seedlings rely on constitutive benzoxazinoid production, at later developmental stages maize responds to biotic challenge with inducible accumulation of a wide range of defense metabolites. Among these, a family of sesquiterpenoids, termed zealexins, is a ubiquitous and often dominant chemical defense found in both maize and teosinte. To understand the genetic, biochemical and functional basis of zealexin defenses, we integrated transcriptional co-regulation patterns, association mapping studies, combinatorial enzyme assays, proteomics, NMR-based structure elucidation, and targeted CRISPR mutants. This revealed at least three gene clusters defining ten zealexin biosynthetic genes (*Zx*) that underlie the production of sixteen different antibiotic metabolites. Depending on the inbreds examined, the maize genome contains varying functional combinations of four nearly identical terpene synthases (abbreviated *Zx*1-4) that yield beta-bisabolene and beta-macrocarpene. Mutual rank analysis of co-expression patterns in maize transcriptional and proteomic atlases highlighted strong correlations between *Zx* terpene synthase genes and a cluster of genes encoding Cyp71Z-family cytochrome P450 enzymes. Accordingly, biochemical assays showed that both beta-bisabolene and beta-macrocarpene are converted to sesquiterpenoid acids by these three functional and highly promiscuous Cyp71Z enzymes, genes for which have been termed *Zx*5-7. Using biparental association mapping, a third gene cluster encoding three Cyp81D-family P450s, *Zx*8-10, were found to be responsible for zealexin desaturation, enabling the production of zealexin B and C series defenses. CRISPR/Cas9-generated *zx*1-4 quadruple mutants lacking detectable zealexins display dramatic increases in *Fusarium* stalk rot and are more susceptible to a range of pathogens. The genetic complexity and pathway redundancy of zealexin biosynthesis ensures that no single gene mutation can compromise production of essential maize defenses, indicating a core role for these defense metabolites in maize biotic resistance.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), UC San Diego start-up funds

T19

## Improving maize NUE through multiplexed genome editing

(presented by Davide Sosso <[dsosso@inari.com](mailto:dsosso@inari.com)>)

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Nitrogen is the most abundantly used fertilizer in agriculture and despite its benefits, it carries both significant production and environmental costs. In this respect, breeding for efficient use of nitrogen (NUE) has become a priority. Inari Agriculture, a recently formed startup, aims to radically transform plant breeding through its Seed Foundry™. As proof of concept, we applied the Seed Foundry™ process to improve Nitrogen Use Efficiency (NUE) in corn through targeted modifications of gene expression. The Inari Seed Foundry™ makes use of custom-developed software and proprietary cell-based assays to build genetic knowledge, and associate sequence polymorphisms and/or specific gene networks to quantitative traits. Once the target sequences are identified, we use our proprietary genome editing toolbox to generate new allelic diversity and introduce this diversity into our elite parental seed. Seed are then delivered to our farm network partners and real-time data are provided back to Inari to improve our genetic knowledge and cast new products. We are now running two iterations of the process with the goal to both optimize nitrogen uptake (NUpE) and utilization (NUtE) efficiency. The first iteration has 6 target genes and the second, more sophisticated approach, involves more than 20 genes. Here, we present our early results on the first iteration, from gene nomination, to guide design and testing, and from genome editing to plant characterization.



T20



## Strigolactone deficient maize dramatically reduces parasitism by the “witchweed”, *Striga*, and reveals other unknown stimulants.

(presented by Jiahn-Chou Guan <[guanjc@ufl.edu](mailto:guanjc@ufl.edu)>)

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Strigolactones (SL) are maize hormones sensed by the parasitic “witchweed”, *Striga*, and required as chemo-signals for germination of its own seeds. *Striga* is a genus of parasitic weeds that widely infest roots of maize in Sub-Saharan Africa, where it decreases maize yield up to 68% or more. A previously-unexplored avenue of *Striga* resistance is offered by a naturally-occurring SL-deficient mutant, *Z. mays carotenoid cleavage dioxygenase 8* (*zmccd8*), which allows little to no production of precursors for SL. The non-transgenic nature of this maize mutant also enhances prospects for its acceptance and use by peoples with diverse cultures. In addition to this potential, our data show that the relatively modest yield penalty in *zmccd8* maize can be countered by using hybrid vigor and other naturally-occurring genetic modifiers. Further, *Striga* seed germination assays (at IITA in Nigeria, Africa) consistently showed that far fewer *Striga* plants attached to roots of *zmccd8* maize or emerged from soil when grown in containers with the mutant host. Although the *zmccd8* mutation offered strong resistance to *Striga*, a residual degree of *Striga* germination and attachment were observed. In addition, alternative bioassays were conducted using *Arabidopsis* seeds that over-expressed the *ShHTL7* (*Striga hermonthica* *Hyposensitive-To-Light 7*) transgene, which is the most sensitive *Striga* SL receptor among other 10 candidates. In these instances, *zmccd8* roots allowed an unexpectedly greater germination relative to wild-type maize roots. We therefore suggest that maize-root exudates other than SL alone trigger the residual *Striga* responses to the SL-deficient maize. Nonetheless, this SL biosynthetic mutant contributes a strong source of potential resistance for combating the devastation to maize by *Striga*.

Gene / Gene Models described: *ZmCCD8*; GRMZM2G446858

Funding acknowledgement: National Science Foundation (NSF)

T21



## **Root metaxylem as a novel target for improved drought tolerance in maize**

(presented by Stephanie Klein <[spk185@psu.edu](mailto:spk185@psu.edu)>)

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Water deficit stress is expected to become more frequent and severe as a result of climate change. Therefore, it is critical to develop drought tolerant crops to generate a global yield capable of sustaining a growing population. We hypothesize that restricted conductance through smaller, more numerous root metaxylem vessels enhances drought tolerance by improving the overall economy of water use. We grew 415 accessions of the Wisconsin Diversity Panel (WiDiv) in the field under well-watered and moderate water stress conditions at the Apache Root Biology Center outside of Willcox, AZ, in 2016. Using shovelomics, root crowns were excavated just before anthesis and washed to collect segments of the fourth node crown root for anatomical phenotyping using laser ablation tomography. We found wide variation in root metaxylem phenotypes in the WiDiv. Under water stress in greenhouse mesocosms using a subset of the WiDiv, we found that lines with smaller metaxylem vessels maintained greater photosynthetic rates and leaf water potentials than lines with larger vessels. To determine the genetic mechanisms underlying variation in metaxylem phenotypes, a genome-wide association study was performed with the WiDiv and a panel of 577,161 single nucleotide polymorphisms (SNPs) identified via RNA-seq. Several significant SNPs were identified under water-stressed and well-watered conditions, and for the plastic response to drought. No SNPs were significant under all conditions, suggesting that genetic mechanisms affecting metaxylem phenotypes under stress and non-stress and for plasticity are separately controlled. Six significant SNPs have been validated through additional screens in the greenhouse as having a strong minor allelic effect on metaxylem phenotypes, including median vessel area and vessel number. The genes where these SNPs reside are also highly expressed in maize root tissues, particularly in the stele. Ultimately, these candidate genes may become novel targets in maize molecular breeding programs.

Funding acknowledgement: United States Department of Agriculture (USDA)

T22 **Assembly and comparative genomic analysis of the maize NAM founders**  
(presented by Matthew Hufford <[mhufford@iastate.edu](mailto:mhufford@iastate.edu)>)

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The 25 founders of the maize Nested Association Mapping (NAM) population represent a highly diverse sampling of modern inbred lines. These lines were each crossed to B73 to create a mapping resource consisting of nearly 5000 Recombinant Inbred Lines (RILs). The NAM RIL population has been used by the maize community to assess the genetic basis of a number of quantitative traits. Analysis of the founder lines *per se* has also contributed to our basic understanding of the diversity and dynamic nature of the maize genome. Up until now, trait mapping and genomic analyses in NAM and its founders have relied on a resequencing approach that can only assess portions of the genome held in common with the B73 reference. However, long-read sequencing, optical mapping, and computational approaches have matured to the point that rapid *de novo* assembly of maize is feasible. Here we present pseudomolecule-level genome assemblies of the maize NAM founders characterized by highly contiguous genic and repetitive space. Based on these gold-quality assemblies, we have conducted comparative genomic analyses revealing substantial, large-scale structural variation across lines. For example, multiple megabase-scale inversions predicted based on suppressed recombination in NAM RIL families have been verified in the assemblies and novel inversions have been documented as well. We have additionally investigated patterns of fractionation across lines, finding lineage-specific patterns of homeolog retention that suggest this process is ongoing. Moving forward, these assemblies will serve as an important resource for the maize community, facilitating an expanded understanding of both the genetic basis of agronomic traits and the complexity of the maize genome.

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

T23

## **Cloning an ear length QTL reveals ethylene as a developmental signal controlling kernel number in maize.**

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Maize is the world's most productive cereal crop, in part due to the development of large ears with many hundreds of kernels. The ear develops by a stereotypical pattern of meristem divisions; the inflorescence meristem produces indeterminate spikelet-pair meristems which branch to form determinate spikelets, which then terminate with the production of florets. Therefore, meristematic activity in the inflorescence determines the number of florets and seeds on the ear. Recent insights have revealed genes important for the number of kernel rows, but control of the number of kernels per row, and ear length, is poorly understood.

In a QTL population, we determined the developmental basis for ear length variation to be due to spikelet potential, not spikelet number per se. In other words, long ear lines do not make more spikelets, but more of the spikelets form seeds. We cloned a QTL, *qEL7*, underlying this trait, and show how it controls ear length. *qEL7* encodes an ethylene biosynthetic enzyme, 1-aminocyclopropane-1-carboxylate oxidase2 (*ACO2*), and we confirmed its function by gene expression, enzyme kinetics assays and transgenic validation. Silencing of *ZmACO2* resulted in a ~20% increase in ear length and similar increase in kernel number per row. We identified a 7 bp indel nearby a *FASCIATED EAR4* binding motif in the *ZmACO2* promoter as the candidate polymorphism controlling *ZmACO2* expression and endogenous ethylene levels. To confirm a role of ethylene, we phenocopied the ear length differences by exogenous ethylene treatments, and found that ethylene induces expression of key developmental regulators, including *BARREN INFLORESCENCE4* and AP2/EREBP transcription factors such as *INDETERMINATE SPIKELET1* and *BRANCHED SHIKLESS1*. Our studies provide direct evidence for a role of ethylene in the regulation of spikelet meristem fate, kernel number and ear length, and provide a tool to improve grain yield by modifying ethylene levels.

Funding acknowledgement: National Science Foundation (NSF)

T24

## **The maize *maternal rough endosperm1 (mre1)* mutant is a parent-of-origin effect locus that disrupts endosperm development**

(presented by Fang Bai <[fbai001@ufl.edu](mailto:fbai001@ufl.edu)>)

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Genomic imprinting in plants is an epigenetic phenomenon by which a subset of genes is expressed in a parent-of-origin-dependent manner. Imprinted gene expression primarily occurs in the endosperm and is thought to influence seed size and embryo development. Recently, we identified a parent-of-origin effect seed mutant, *maternal rough endosperm1 (mre1)* through screening rough endosperm mutants from the UniformMu transposon-tagging population. Reciprocal crosses between heterozygous mutants and inbred plants show a rough kernel phenotype only when *mre1* is inherited from the female parent. Mutant *mre1* kernels have a general delay in endosperm development with smaller starchy endosperm cells, delayed basal endosperm transfer cell layer (BETL) development, and delayed accumulation of starch granules. Crosses of *mre1* with the *pVP1::GUS* reporter and qRT-PCR confirmed reduced expression of endosperm cell type markers in the mutant. RNA-seq analysis from hybrid crosses of *mre1* /+ X B73 found an increase in the number of genes with parental biased expression, consistent with a delay in endosperm differentiation. Molecular mapping located *mre1* on chromosome 4. Whole genome sequencing revealed a candidate gene that has a point mutation causing a premature termination codon. An additional mutant allele is available from the UniformMu reverse genetics resources to verify if the *mre1* candidate causes the maternal-effect phenotype.

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

T25

**The *Maize LINC KASH AtSINE-like2 (MLKS2)* gene encodes an ARM domain KASH protein that tethers the nucleus to actin and is required for normal development and meiotic chromosome segregation.**

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The LINC (Linker of Nucleoskeleton to Cytoskeleton) complex is an essential multi-protein structure spanning the nuclear envelope. It connects the cytoplasm to the nucleoplasm, functions to maintain nuclear shape and architecture; and regulates chromosome dynamics during cell division. We recently identified and characterized 22 genes encoding the maize LINC complex proteins (Gumber et al., JCS, 2019). Here we present new findings on one of these, the *Maize LINC KASH AtSINE-like2 (MLKS2)* gene. MLKS2 encodes a KASH protein with a canonical single transmembrane domain and a C-terminal V-P-T sequence. The MLKS2, like its eudicot counterpart, AtSINE, also has N-terminal armadillo repeats (ARM), predicted to mediate protein-protein interactions. Using a heterologous expression system, we showed that GFP-MLKS2 is targeted to the nuclear periphery, colocalizes with actin in the cell cortex, and requires both KASH and ARM domains for nuclear anchoring. Genetic and phenotypic analysis of two newly characterized transposon-tagged alleles of MLKS2 revealed multiple different phenotypes in various cell-types or tissues, including root hair (nuclear morphology and position), stomatal complex (subsidiary cell development), male meiocytes (bouquet, nuclear architecture, chromosome missegregation), and pollen (viability). Given the importance of the nuclear envelope and the meiotic SUN belt in chromosome behavior and segregation, we examined the effect of MLKS2 mutation on meiosis using 3D cytology. We found that MLKS2 mutants showed a reduction in perinuclear actin, partial telomere bouquet, and unusual clumping of chromosomes at late prophase, followed by evidence of chromosome missegregation at meiosis I and II. Pollen viability was also notably reduced in the MLKS2 mutants. These findings place MLKS2 in the meiotic chromosome segregation pathway, likely mediated by a chain connecting chromosomes to actin through SUN-MLKS2 LINC complex. This and other developmental defects reveal the importance of SINE-type KASH proteins in basic cellular processes required for normal plant growth and fertility.

Gene / Gene Models described: *mlks2*; Zm00001d052955

Funding acknowledgement: National Science Foundation (NSF)

T26

## Paramutation at the maize *pl1* locus is manifest by a developmentally-essential CHD3 nucleosome remodeler

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In maize, paramutations result in directed and meiotically-heritable regulatory changes of certain *purple plant1* alleles<sup>1</sup> which encode a R2R3 MYB protein required for anthocyanin production<sup>2</sup>. A strongly-expressed *P11-Rhoades* allele is suppressed in trans when combined with a transcriptionally and post-transcriptionally repressed *P11-Rhoades* allele (denoted *Pl'*), and both alleles are sexually transmitted in a repressed state. Mutant screens have identified at least sixteen loci whose functions are *required to maintain repression (rmr)* of *Pl'*<sup>3,4</sup>. All known RMR proteins mediate 24 nucleotide (24nt) RNA biogenesis<sup>4,5,6,7,8</sup>, and four are putative orthologs of *Arabidopsis* proteins that facilitate repressive chromatin modifications. Here we introduce four EMS-derived recessive alleles defining the *rmr12* locus. Unlike other *rmr*-type mutations found to date<sup>4,5,7,8,9,10</sup>, *rmr12* mutants display a unique set of leaf, inflorescence, and pollen defects that highlight a novel mechanistic connection between paramutation and developmental gene control. We used position-based cloning to discover that *rmr12* encodes a chromodomain helicase-DNA binding3 (CHD3) protein whose closest *Arabidopsis* ortholog, PICKLE, influences nucleosome homeostasis<sup>11,12</sup> presumably through its ability to translocate nucleosomes along a DNA substrate<sup>13</sup>. Genetic data indicates that RMR12 is not responsible for maintaining the epigenetic feature typifying *pl1* paramutation thus leading to models in which RMR12 manifests repression through altering nucleosome / DNA interactions in response to a meiotically-heritable feature.s

**1** Hollick *et al.* 1995 *Genetics* 141, 709 | **2** Cone *et al.* 1993 *Plant Cell* 5, 1795 | **3** Hollick and Chandler 2001 *Genetics* 157, 369 | **4** Hale *et al.* 2007 *PLoS Biol.* 5, 2156 | **5** Erhard *et al.* 2009 *Science* 323, 1201 | **6** Nobuta *et al.* 2008 *PNAS* 105, 14958 | **7** Stonaker *et al.* 2009 *PLoS Genet.* 5, e1000706 | **8** Barbour *et al.* 2012 *Plant Cell* 24, 1761 | **9** Dorweiler *et al.* 2000 *Plant Cell* 12, 2101 | **10** Parkinson *et al.* 2007 *Dev. Biol.* 308, 462 | **11** Ogas *et al.* 1999 *PNAS* 96, 13839 | **12** Carter *et al.* 2018 *Plant Cell* 30, 1337 | **13** Ho *et al.* 2013 *Biochim. Biophys. Acta* 1829, 199

Gene / Gene Models described: *rmr12/chd3a*; Zm00001d045109

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Syngenta, OSU Center for Applied Plant Sciences



T27

## **A silk-expressed pectin methylesterase confers cross-incompatibility between wild and domesticated strains of *Zea mays***

(presented by Yongxian Lu <[yxlu@carnegiescience.edu](mailto:yxlu@carnegiescience.edu)>)

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A central problem in speciation is the origin and mechanisms of reproductive barriers that block gene flow between sympatric populations. In sexually reproducing plants, reproductive barriers exist at different stages during reproduction, including pre-pollination, post-pollination and post-fertilization. Post-pollination barriers depend on interaction between the male gametophyte (pollen) and the cells of the female reproductive organs (stigma, style, and ovule).

In *Zea mays*, three haplotypes, *Gametophyte factor1-s* (*Ga1-s*), *Gametophyte factor2-s* (*Ga2-s*), and *Teosinte crossing barrier1-s* (*Tcb1-s*) at three different loci confer Unilateral Cross-Incompatibility by arresting non-self growing pollen tubes. While *Ga1-s* and *Ga2-s* are widespread in domesticated maize, *Tcb1-s* is almost exclusively found in wild teosinte populations. Despite being members of the same species, some strains of wild teosinte maintain themselves as a distinct breeding population by blocking fertilization by pollen from neighboring maize plants. These teosinte strains may be in the process of evolving into a separate species, since formation of reproductive barriers is a critical step in speciation. These teosinte strains typically carry the *Tcb1-s* haplotype. *Tcb1-s* contains a female barrier gene that blocks non-self-type pollen and a male function that enables self-type pollen to overcome that block. With genetic and genomic approaches, here we show that the *Tcb1-female* barrier gene encodes a Pectin Methylesterase<sup>38</sup> homolog, implying that pollen cell wall modification is a key cellular mechanism by which these teosinte females reject foreign but closely related pollen. Cloning of this female barrier gene in *Zea mays* represent a major advance in speciation research and opens up exciting working hypotheses to test. Agriculturally, this work may also help 1) managing specialty crop populations by preventing pollen contamination; 2) facilitating development of breeding tools to enrich crop genetic pools by backcrossing crops to their ancestors for the purposes of yield increase or enhanced stress resistance.

Funding acknowledgement: National Science Foundation (NSF)

T28

**Evidence of widespread gene-distal cis-regulatory elements in the maize genome**(presented by Bill Ricci <[william.ricci@uga.edu](mailto:william.ricci@uga.edu)>)Full Author List: Ricci, William A<sup>1</sup>; Lu, Zefu<sup>2</sup>; Ji, Lexiang<sup>3</sup>; Marand, Alexandre P<sup>2</sup>; Noshay, Jaclyn M<sup>4</sup>; Galli, Mary<sup>5</sup>; Mejía-Guerra, María K<sup>6</sup>; Buckler, Edward S<sup>6 7 8</sup>; Gallavotti, Andrea<sup>5</sup>; Springer, Nathan M<sup>4</sup>; Schmitz, Robert J<sup>2 3</sup>; Zhang, Xiaoyu<sup>1</sup><sup>1</sup> Department of Plant Biology, University of Georgia, Athens, GA, 30602<sup>2</sup> Department of Genetics, University of Georgia, Athens, GA, 30602<sup>3</sup> Institute of Bioinformatics, University of Georgia, Athens, GA, 30602<sup>4</sup> Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN, 55108<sup>5</sup> Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, 08854<sup>6</sup> Section of Plant Breeding and Genetics, Cornell University, Ithaca, NY, 14853<sup>7</sup> Institute for Genomic Diversity, Cornell University, Ithaca, NY, 14853<sup>8</sup> USDA-ARS, R. W. Holley Center, Cornell University, Ithaca, NY, 14853

Mammalian genomes contain abundant transcriptional cis-regulatory elements (CREs) that interact with genes over long distances via the formation of chromatin loops. Such long-distance interactions are critical for the precise control of transcription. In contrast, the extent and importance of gene-distal CREs within plant genomes remains an outstanding question. Several lines of evidence have casted doubt on their existence in plants: the compact genome of the model plant *Arabidopsis thaliana* contains few long-range euchromatic loops; the H3K4me1 histone modification—which marks a majority of the gene-distal CREs in mammalian genomes—is uncommon in plant intergenic space; and plants lack homologues of the loop-mediating protein CTCF. However, genetic evidence suggests that gene-distal CREs exist within the less compact genome of *Zea mays* (maize): the CREs of several important maize genes have been mapped to loci tens of kilobases away from their target genes and a large number of functional sequence variants reside within the gene-distal intergenic space. The prevalence, sequence and chromatin attributes, transcriptional regulatory behavior, and mechanisms of action of these hypothesized gene-distal CREs remain largely unexplored. Here, we provide evidence supporting the widespread existence of gene-distal CREs in maize. We show that gene-distal accessible chromatin regions (ACRs) display functional sequence enrichment and unique chromatin attributes that predict their regulatory functions. Unlike for mammalian enhancers, no common histone modification is shared by all distal ACRs. Many distal ACRs physically interact with genes and individual distal ACRs often interact with multiple genes. Additionally, many distal ACRs display transcriptional enhancer activity. These results uncover a widespread mode of transcriptional regulation in plants and suggest that the control of transcription by gene-distal CREs may be a common feature among eukaryotic genomes.

Funding acknowledgement: National Science Foundation (NSF)

T29



## **Three-dimensional chromatin interactions reveals the functional maize genome**

(presented by Yong Peng <[pengyong@webmail.hzau.edu.cn](mailto:pengyong@webmail.hzau.edu.cn)>)

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Chromatin loops connecting regulatory elements to their target genes are the bridges between transcriptional regulation and phenotypic variation in mammals. However, spatial organization of regulatory elements and its impact on gene expression in plants remains unclear. We characterized epigenetic features of active promoter proximal regions and candidate distal regulatory elements to construct high-resolution chromatin interaction maps for maize via long-read chromatin interaction analysis by paired-end tag sequencing (ChIA-PET). The maps indicate that chromatin loops are formed between regulatory elements, and that gene pairs between promoter proximal regions (proximal-proximal interaction, PPI) tend to be co-expressed. The maps demonstrated the topological basis of quantitative trait loci which influence gene expression and phenotype. Many promoter proximal regions are involved in chromatin loops with distal regulatory elements, which regulate important agronomic traits. Collectively, these maps provide an unprecedented high-resolution view of 3D maize genome architecture, and its role in gene expression and phenotypic variation.

Funding acknowledgement: National Science Foundation (NSF), National Key Research and Development Program of China

T30

## **TR1 knobs become motile neocentromeres in the presence of a kinesin-14-like motor protein encoded on Ab10**

(presented by Kyle Swentowsky <[kws67291@uga.edu](mailto:kws67291@uga.edu)>)

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The maize Abnormal Chromosome 10 (Ab10) haplotype comprises a classical meiotic drive system which exploits the asymmetric cell division that forms the megaspore to favor its own inheritance over normal chromosome 10. In the presence of Ab10, all knobs in the genome become motile neocentromeres during meiosis, and chromosomes with knobs preferentially segregate into the daughter cell that gives rise to the gametophyte. Since Ab10 is geographically widespread and causes segregation distortion in large, gene-rich regions, it has strongly impacted maize evolutionary history. Knobs are composed of two distinct tandem repeat arrays termed 180bp and TR1. We recently showed that Ab10 encodes a kinesin-14 protein family called KINDR that localizes only to 180bp neocentromeres during meiosis. TR1 repeats undergo dramatic neocentromere activity but their motility cannot be explained by KINDR. Here we report the identification of a new kinesin-14 protein, TRKIN (TR1 KINESIN) that is encoded on Ab10 and another chromosome 10 haplotype, K10L2, that also displays TR1 neocentromeres. The Trkin gene structure is peculiar since the 1.6kb CDS stretches over 120kb of genomic sequence, with large introns preceding pairs of exons that are separated by much smaller introns. Trkin is barely homologous to its closest homologs even in its motor domain. TRKIN is a functional minus end-directed motor in vitro and localizes specifically to TR1 knobs during meiosis. Furthermore, a related haplotype called Ab10-II, which does not encode a full-length TRKIN protein, shows 180bp but not TR1 neocentromere activity. Together these data strongly suggest that TRKIN provides the motile force for TR1 neocentromeres.

Funding acknowledgement: National Science Foundation (NSF)

T31

## Epigenetic perturbation of male meiosis in *Zea mays*

(presented by Benjamin Berube <[bberube@cshl.edu](mailto:bberube@cshl.edu)>)

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Meiotic recombination is a fundamental evolutionary driver and an indispensable tool for agricultural breeding. The generation of novel allele combinations is essential for the genetic diversity of populations and the application of agriculturally valuable traits. However, recombination events are not evenly distributed along the chromosomes, making it extremely difficult to disrupt parental haplotypes in regions of limited recombination and directly hindering applied breeding efforts. Thus, it is of primary interest to better understand the mechanisms by which meiotic recombination and subsequent gametogenesis are regulated. Epigenetic marks, including DNA methylation, small RNAs, and chromatin marks, have been increasingly recognized as essential for proper reproductive fidelity and genome integrity. However, the manner in which epigenetic regulation can shape the meiotic environment is less well appreciated. Here, we actively perturb male meiotic development using DNA methyltransferase mutants in order to better understand how epigenome dynamics contribute to proper meiotic progression.

By coupling single-cell sequencing approaches with statistical modeling of recombination breakpoints, we can assay the direct effects of these mutants on germ cell populations. To further emphasize the general utility of this approach, we are also dissecting the genetic components of a novel meiotic drive system in *Zea mays*.

Funding acknowledgement: National Science Foundation (NSF), Howard Hughes Medical Institute (HHMI)

T32



## **More references, more questions: Limitations in maize annotations that leads to different representations of gene models across maize reference genomes**

(presented by Patrick Monnahan <[pmonnaha@umn.edu](mailto:pmonnaha@umn.edu)>)

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Advances in sequencing technologies have lead to the release of numerous maize reference genomes with many more to come in the near future. These technological advances have not only aided in improving the quality of each assembly but allowed researchers to implement new methods for annotating genes across each genome. While each of these new genomes shares significant portions of synteny between each other, the annotated structure of gene models within these regions can differ. To investigate this issue, we compared gene annotations of five reference genomes (B73, Mo17, PH207, PHB47, and W22) to identify gene models that are represented as a single gene in one reference genome and represented as multiple distinct genes within another or vice versa. On average we find that 700 genes are classified as split/merged in pairwise comparisons. To determine which state (i.e. one gene or multiple genes) is biologically correct we incorporated RNAseq data from 10 tissues throughout development as well as comparative genomics with other species including Arabidopsis. The methods we have developed require minimal human interaction and can be applied to future assemblies to aid in annotation efforts.

Funding acknowledgement: National Science Foundation (NSF)

T33

**Meiosis-associated argonaute (MAGO) proteins are necessary for protecting the germline from misregulated transposable elements in maize**  
(presented by Robert Maple <[r.maple@warwick.ac.uk](mailto:r.maple@warwick.ac.uk)>)

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Plants do not specify their germline until late in their life cycle. Hence, the plant germline is normally specified from terminally differentiated somatic cells, though the precise mechanism(s) are unknown. We have found that male gametogenesis in maize is associated with the accumulation of distinct 21-nt phased small-interfering RNAs (phasiRNAs) generated by meiosis-associated argonaute (MAGO) proteins. These proteins accumulate in the epidermis of pre-meiotic anthers and in developing meiocytes, are necessary for meiocyte development and mutants display chromosomal defects and male infertility. Furthermore, we have found that a distinct class of Long Terminal Repeat (LTR) retrotransposons are activated in the male germline of MAGO mutants upon heat stress. Our data suggests that MAGO proteins and reproductive phasiRNAs play important roles in protecting the germline from transposable element misregulation during environmental stress conditions.

Funding acknowledgement: BBSRC, Biogemma



T34

## Investigation of gene regulatory network of maize endosperm development (presented by Hao Wu <[haowu@iastate.edu](mailto:haowu@iastate.edu)>)

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NKD1, NKD2 and OPAQUE-2 (O2) are three important transcription factors (TFs) that control gene networks associated with maize endosperm development. To further explore their functions, a family of *nkd1*, *nkd2* and *o2* homozygous mutants (including all single, double and triple mutant combinations and wildtype control) was generated. They manifested diverse phenotypes in endosperm. RNA sequencing-based differential gene expression analysis indicated a dynamic influence of the three TFs on downstream genes at 8, 12 and 16 DAP. Weighted gene co-expression network analysis (WGCNA) identified hub TF genes significantly correlated with at least one of the three primary TFs suggesting they may be central regulators of their sub-networks (modules) that functions in specific biological processes at different development time points. 34 hub TF genes were chosen for further investigation and DNA affinity purification sequencing (DAP-seq) was performed to identify their putative targets. Binding motifs and putative targets were enriched for 12 of the hub TFs. Some of the 12 TFs (e.g. BZIP100, GBF1, EREB117) are putative direct targets of NKD1, NKD2 or O2, and they also regulate a broad range of downstream genes, including other TFs, forming a hierarchical gene network architecture. Gene ontology (GO) term enrichment analysis of the putative target genes indicated specific functions for several TFs and their modules. For example, EREB117 target genes were enriched for GO terms related to plastids, consistent with the GO terms of its module that was positively correlated with NKD1 at 8 DAP. Thus, NKD1 and EREB117 may hierarchically regulate metabolic processes involving plastids in early endosperm development. In all, this analysis provided deeper insight into NKD1, NKD2 and O2 gene regulatory network in endosperm development.

Funding acknowledgement: National Science Foundation (NSF)



## Decoding the transcriptional regulatory atlas of the maize leaf

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Complex traits related to the structure and physiology of the leaf are determinants of maize yield. Important genes have been identified through quantitative genetic studies, evolutionary comparative analysis, and transcriptomes across tissues and developmental stages. Yet, the architecture of the gene regulatory network controlling the functioning of the maize leaf is unknown. In this study we used a **novel ChIP-seq** approach to catalog the *in vivo* binding of **104 transcription factors (TFs)** that are highly expressed in leaves. The resulting **2,162,941** highly reproducible peaks represent a non-overlapping set of 144,890 regulatory loci. This collection highly overlaps with open chromatin regions (*i.e.*, 99% of ATAC-seq peaks), and show enrichment for expression quantitative trait loci (eQTLs).

Peak information was used to (1) generate machine learning models of TF-binding, and (2) for network analysis to characterize the gene regulatory network that governs the phenotype of the maize leaf. We trained machine learning models to characterize the sequence preferences for the binding of each TF individually (accuracy ~85%) and to model TF-partnerships and co-binding (accuracy > 90%). Clustering of the TFs binding sequence preferences closely recapitulates the evolutionary similarity among TFs. We observed some TFs being differential predictors of proximal and distal binding, and identified a group of TFs highly predictive across the co-binding contexts of the other TFs. Mutants of *Arabidopsis* orthologs for these hubs show enrichment in observable phenotypes. A community analysis revealed a highly modular regulatory network, with the communities presenting non-random enrichment of GO terms. We observed a continuous range of community specialization, ranging from some including a large number of TFs, to communities with few/one TF highly specialized in few biological processes. The atlas and models presented here will be instrumental to interpret sequence variation in the light of its regulatory function.

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

## **Poster Abstracts**

P1 

### **Next generation data management**

(submitted by Lisa Harper <[lisa.harper@ars.usda.gov](mailto:lisa.harper@ars.usda.gov)>)

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We are nearing at a time in history where we will have the potential to capture and curate almost all published maize data. Imagine if every piece of published biological data is available from easy-to-find and well-organized resources; the data are accurately described and available in accessible and standard formats; the experimental procedures, samples and time points are all completely documented; and researchers can find answers to any question about the data with just a few mouse clicks. This could affect all of our research in ways we cannot even yet imagine. However, the amount of published data has risen exponentially while curator person-hours have remained constant. To facilitate “next generation curation”, we only need to make a few simple changes to our workflows to have enormous positive effects in data viability. In this talk, we will outline how to achieve this, and ways that MaizeGDB can help.

Funding acknowledgement: United States Department of Agriculture (USDA)

P2 

### **Using MaizeMine for genomic data integration and meta-analysis**

(submitted by Jack Gardiner <[jack.m.gardiner@gmail.com](mailto:jack.m.gardiner@gmail.com)>)

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The availability of high-throughput genomic technologies has accelerated the generation of massive quantities of genomic datasets. Maize researchers often wish to perform comparative analysis between their own datasets and published or publicly available data. MaizeMine (<http://maizemine.maizegdb.org>), MaizeGDB's data mining warehouse, enables researchers without scripting skills to integrate their data with publicly available data and perform meta-analysis. The MaizeMine List tool allows users to upload identifiers to create custom lists, perform set of operations such as unions and intersections, and execute template queries with lists. Users can easily compare their results with published results by uploading genomic coordinates or identifiers. MaizeMine uses the InterMine data warehousing system to integrate genomic sequences from the B73\_RefGen\_v3 and B73\_RefGen\_v4 genome assemblies, three sets of gene annotations (AGPv3, AGPv4, RefSeq), Gene Ontology (GO), protein annotations (UniProt), protein families and domains (InterPro), homologs (Ensembl Compara), and pathways (CornCyc, KEGG, Plant Reactome). In our most recent update of MaizeMine (v1.3), we have added data sets for a SNP array (Illumina SNP50), whole genome EMS mutagenesis sites, three insertional mutagenesis collections (Brutnell AcDs, Barker\_Mu Illumina, and McCarty Uniform Mu), MaizeGamer annotations, and root and shoot transcriptional start sites. MaizeMine also provides pre-computed variant effects and expression levels based on RNA-seq data from the Zea mays Gene Expression Atlas (NCBI BioProject PRJNA171684). Database cross references facilitate easy gene identifier conversion between AGPv3, AGPv4 and RefSeq. MaizeMine provides simple and sophisticated search tools, including a keyword search, built-in template queries with intuitive search menus, and a QueryBuilder tool for creating custom queries.

Funding acknowledgement: United States Department of Agriculture (USDA)

P3 

### **MaizeGDB 2019: the maize multi-genome genetics and genomics database.**

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MaizeGDB, the Maize Genetics and Genomics Database, has expanded to support the sequenced genomes of many maize inbred lines in addition to the B73 reference genome assembly. Curation and development efforts have targeted high-quality datasets and tools to support maize trait analysis, germplasm analysis, genetic studies, and breeding. MaizeGDB hosts a wide range of data including recent support of new data types including genome metadata, RNA-seq, proteomics, synteny, and large-scale diversity. To improve access and visualization of data types several new tools have been implemented to: access large-scale maize diversity data (SNPiversity), Compare and download gene expression data (qTeller), visualize pedigree data (Pedigree Viewer), link genes with phenotype images (MaizeDIG), and enable flexible user-specified queries to the MaizeGDB database (MaizeMine). MaizeGDB also continues to be the community hub for maize research, coordinating activities and providing technical support to the maize research community. Here we report the changes MaizeGDB has made within the last several years to keep pace with recent software and research advances, as well as integrating and representing pan-genomic data sets made possible through better and less expensive sequencing technologies. MaizeGDB is accessible online at <https://www.maizegdb.org>.

Funding acknowledgement: United States Department of Agriculture (USDA)

P4 

### **3D time lapse analysis reveals multiscale relationships in contrasting maize root architectures**

(submitted by Christopher Topp <[ctopp@danforthcenter.org](mailto:ctopp@danforthcenter.org)>)

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To understand how an organism's phenotypic traits are conditioned by genetic and environmental variation is a central goal of biology. Root systems are one of the most important but poorly understood aspects of plants, largely due to the three-dimensional, dynamic and multiscale phenotyping challenge they pose. A critical gap in our knowledge is how root systems build in complexity from a single primary root to a network comprised of thousands of roots that coordinate functions to compete for ephemeral and heterogeneous soil resources. We used time lapse 3D imaging and mathematical modelling to assess root architectures of two maize inbred genotypes (B73 and Mo17) and their hybrid as they grew in complexity from few to many roots. Genetically driven differences in the size of the root branching zone and lateral branching densities along a single root, combined with differences in peak growth rate and the relative allocation of carbon resources to new versus existing roots, manifest as sharply distinct global root architectures over time. 3D imaging of mature field-grown root crowns, the nexus of the root system, showed that these genetic differences in seedling architectures persisted throughout development and across environments, contrary to the oft-cited preeminence of environmental versus genetic factors in root architecture. The work connects individual and system-wide scales of root growth dynamics, providing the means for empirically-driven multi scale modelling approaches that could eventually predict genetic variation for complex root shapes and their functions.

Funding acknowledgement: National Science Foundation (NSF)

P5 

## A fast computational pipeline for *de novo* assembly of plant genomes from long reads

(submitted by Evan Rees <[err87@cornell.edu](mailto:err87@cornell.edu)>)

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The advent of the genomics era has seen an explosion of genome assemblies, dominated by prokaryotes and vertebrates but recently permeating the plant kingdom. These assemblies have largely been generated using a combination of high-depth paired-end and mate-paired short Illumina reads, and optical maps. Drawbacks of this overall approach include the considerable cost of computing time, materials, and labor, along with the difficulty of resolving large, repeat-rich eukaryotic genomes. Historically hindered by high cost per basepair and low accuracy when compared with Illumina, recent improvements have established long-read sequencing technologies as a viable supplement to existing methodologies. Using current methods, Oxford Nanopore Technologies' MinION flowcells routinely yield in excess of 10 Gbp of reads with N50 > 30 Kbp and median PhredQ > 8. We have leveraged this technology to sequence and *de novo* assemble the maize P39 genome to contig level from moderate coverage (~30X) of long reads for structural resolution, and low coverage (~10X) of Illumina short-reads for sequence accuracy. Our assembly displays high-contiguity and completeness, with N50 > 400 Kbp and >90% of embryophyta BUSCOs present in single-copy. We have developed our computational pipeline using open-source tools designed for long, error-prone sequence data, based around the ultra-fast wtdbg2 assembler, minimap2 aligner, Racon consensus module, and Pilon assembly improvement tool. Our pipeline is implemented as a Docker container to ensure portability, reproducibility, and customizability, and has been additionally tested in *Tripsacum floridanum*, *T. dactyloides*, *Urelytrum digitatum*, *Miscanthus junceus*, and *Cucumis melo* with comparable results. On a computing node with 112 cores and 512 Gb RAM, we are able to produce such assemblies in less than one day. These assemblies will facilitate our efforts in exploring genetic diversity in the Andropogoneae tribe, with a particular focus on mapping cold-tolerance QTL in *Tripsacum*, the sister-genus of *Zea*.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), US-Israel Binational Agricultural Research and Development Fund (BARD), Bill & Melinda Gates Foundation

P6 

## **A genome-wide association study of maize inflorescence traits and their plasticity under nitrogen stress**

(submitted by Brandi Sigmon <[bsigmon2@unl.edu](mailto:bsigmon2@unl.edu)>)

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Nitrogen is an essential constituent for proper growth and development in maize. As such, nitrogen application is an indispensable part of crop production. Deficiencies in nitrogen can lead to physiological and morphological changes in maize, which in turn lead to decreases in yield. There is a wide range of variation in response to nitrogen deficiency across genetically diverse maize accessions. Some of this variation may be due to differences in root-rhizobium interactions from differences in root exudates and the recruitment of beneficial microbes in individual maize genotypes. To better assess the phenotypic impact of nitrogen stress on maize inflorescences under field conditions, we grew out the maize 282 diversity panel under normal and nitrogen deficient conditions and phenotyped for flowering time, inflorescence length, branch zone length, spike length, primary branch number in maize tassels, as well as, ear length, ear width, row number, ear fill, kernel abortion, and kernel dimensions in maize ears. A Genome-Wide Association Study (GWAS) was then performed to identify trait associated SNPs for these inflorescence traits. Candidate loci include genes involved in stress response, plant growth and development, and root growth. These data will then be combined with rhizobiome results to determine if the phenotypic plasticity in response to nitrogen stress is correlated to differences in soil and root microbial composition, abundance, and activity, as well as, drone imagery and leaf spectral measurements to assess how effectively maize reproductive stress severity can be estimated from these proxy data types. In future, these data will be used to optimize maize germplasm for improved reproductive resilience under low-nitrogen conditions.

Funding acknowledgement: National Science Foundation (NSF), Nebraska EPSCoR

P7 

## **A novel solution to describe and manage maize genomic variation for high resolution genotyping**

(submitted by Paul Chomet <[paul.chomet@nrgene.com](mailto:paul.chomet@nrgene.com)>)

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Next Generation sequencing technologies have opened the door to multiple genome analyses and an increased understanding of the variations present in populations. To date, most of the germplasm analyses have relied on the comparison of sequence reads to one reference genome assembly, limiting our understanding of genomic variation. NRGene has developed novel analytics and approaches to efficiently describe the relevant variation across germplasm using sequence-based haplotypes. These analytics along with new sequencing library methods from iGenomX and low-cost Illumina based sequencing have enabled a cost effective, high resolution, whole genome sequence method for genotyping maize populations. A genotyping example based on ultra-low sequence coverage using this method will be presented.

**P8**

## **A novel, evidence-weighted pipeline for improving maize gene structure annotations**

(submitted by Arun Seetharam <[arnstrm@iastate.edu](mailto:arnstrm@iastate.edu)>)

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The most recent version of the maize B73 genome, Version 4, was released in 2016 (Jiao et al., 2017) and included a primarily de-novo gene annotation set, utilizing grass and Arabidopsis protein and transcript data as well as maize RNA-seq and Iso-Seq reads as inputs for the MAKER-P annotation pipeline (Campbell et al., 2014). Shortly after this annotation was released, two additional annotations of the V4 genome were generated independently by NCBI and EvidentialGene (Gilbert, 2016). These groups used completely separate, in-house annotation algorithms. In many cases, these independent Version 4 maize annotations have conflicting models for the same genes. Additionally, each Version 4 annotation set has conflicts with annotations based on previous versions of the B73 genome. Here we present a new pipeline that utilizes the substantial transcriptomic data available in public databases to simultaneously improve existing gene structures, infer new genes with weight given primarily to evidence, and predict any remaining unidentified genes in B73 using the ab initio predictor BRAKER2. All transcripts are weighted based on the extent of expression evidence from public datasets and finalized annotations will be ranked. Preliminary results of this pipeline applied to Arabidopsis, using 1160 SRA runs, detected roughly 2,500 novel transcripts. We are using this approach to help unify multiple versions of annotations in maize and improve their utility for the broader community.

Funding acknowledgement: National Science Foundation (NSF)

**P9**

## **A survey of the maize-associated microbiota in georgia**

(submitted by Jason Wallace <[jason.wallace@uga.edu](mailto:jason.wallace@uga.edu)>)

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The bacteria and fungi that associate with crop plants have great potential for benefiting global agriculture, but our understanding of how they assemble and what affects them is still very rudimentary. To identify some of the major drivers of these microbial communities (“microbiota”), we sampled maize-associated microbial communities at >30 field sites across the state of Georgia. All samples were collected during the 2018 growing season, with collections occurring at 600 and 1400 GDDs (roughly corresponding to cob initiation and pollination, respectively). Bulk soil, rhizosphere, root, and stalk communities were surveyed by 16s amplicon sequencing. Microbial community summary statistics and composition were compared with soil physiochemical properties, growth stage, and farmer-supplied agronomic data to identify the primary drivers of each microbial community.

Funding acknowledgement: Georgia Agricultural Commodity Commission for Corn



P10 

## A system for managing maize research crops

(submitted by Toni Kazic <[kazict@missouri.edu](mailto:kazict@missouri.edu)>)

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Planning, managing, and collecting data from research crops are labor-intensive tasks. Each laboratory and experiment has its own goals and methods, and these can change with every planting. I have built a flexible system that reduces manual effort and can be easily changed as one's needs evolve. It supports genetics, but could be adapted to breeding programs if needed. The system has been tested and refined with multiple generations of students over the last twelve years. The system includes five components.

1. *Chloe*: a set of Perl scripts to process raw data; generate tags, labels, and barcodes; and performs miscellaneous crop and inventory management tasks. Chloe is one of the many titles of Demeter, meaning "young green shoot".
2. *Demeter*: a Prolog database for crop, genetic, sample, and phenotype data; associated data provenance; and code for pedigree generation, crop planning, and management of field work.
3. Spreadsheets for data collection in the field using smart phones or tablets and small barcode scanners.
4. Cloud services for data transfer from field to server, and secondary storage of raw data.
5. Robust protocols and equipment for plant tagging and the collection and transfer of data.

All code relies entirely on open-source code that run on all desktop platforms. We use spreadsheets on smart phones and tablets to collect the tabular data using small hand-held barcode scanners. The cloud service Dropbox is used to transfer spreadsheet data from the phones to a server and provides secondary storage of the raw data. After manual inspection and correction of the raw data (for example, for failed barcode readings), data are processed and deposited in Demeter. Code and documentation [are on GitHub](#). The code is currently released under the terms of the GNU Affero General Public License v3.0 license.

Funding acknowledgement: National Science Foundation (NSF)

P11

## A web server for Helitron identification

(submitted by Jaydon Lynch <[lynchj21@montclair.edu](mailto:lynchj21@montclair.edu)>)

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User-friendly web application made for Helitron scanning has yet to be created since the current tools are command line based. We've developed a pre-release website that will act as a template for online Helitron prediction. Primary features of the site will include asynchronous file upload of data up to 1 Mb and Helitron prediction output straight to the web application. The file uploaded by all users are handled by listeners then parsed before sending to server made for prediction Helitrons. In order to achieve this our web interface calls a developed Helitron application programming interface, or API, which then returns a prediction back to the site for the user to see. Our API was developed utilizing an artificially intelligence Helitron predictor that is constantly learning. The web server will automate several tasks via communication with the command line version. We adopted the d3js HTML5 graphic library to dynamically generate interactive graphs with user data as well as genome-wide resources. Each task submission is assigned with a unique RID (results ID). User data are kept private and only accessible via the RID prompted to users right after submission. Tasks normally run several minutes, while users can check the status of their tasks with RID. A help page explains different modes and parameters of the server. The web server is developed using modern HTML5 and JavaScript. No programs or plugins need to be installed. This web interface aims to solve that issue by combining a specially designed user experience (UX) and user-interface (UI) with high powered artificial neural networks wrapped up into a well-developed web API to process data quickly and efficiently.

Funding acknowledgement: National Science Foundation (NSF)

P12 

## Accessing MaizeCODE data via SciApps

(submitted by Zhenyuan Lu <[luj@cshl.edu](mailto:luj@cshl.edu)>)

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MaizeCODE, a project for an initial analysis of functional elements in the maize genome, has assayed five tissues of four maize genomes (B73, NC350, W22, TIL11) for RNA-seq, Chip-seq, Rampage, small RNA, and MNase (outside collaboration). MaizeCODE is committed to open accesses and reproducible science based on the FAIR data principles, providing both human and machine access to the data. All raw data has been submitted to NCBI short read archive (SRA) using the SRA submission pipeline in the CyVerse Discovery Environment (DE). Through the submission process, all experimental metadata are stored in the iRODS based Data Store of CyVerse and used for automating the primary analysis on SciApps (<https://www.SciApps.org>), a cloud based bioinformatics workflow platform. SciApps organizes both replicates (and controls if available) of each assay as one experiment (or a workflow with the unique id), which represents an entity that chains raw data, analysis results, experimental metadata, and computational metadata together. The primary analysis includes quality control (QC), alignment to the reference genome, filtering, quantification (e.g. for gene expression), and peak calling (if needed). SciApps provides both a Graphical User Interface (GUI) and a RESTful API for users to check QC results, process new data, and reproduce existing analysis on the Texas Advanced Computing Center (TACC) cloud. All raw data and results are staged in the CyVerse Data Store so that secondary analysis, e.g. differential expression analysis between two tissues, can be done in minutes with prestaged results. For Visualization, SciApps supports visualizing the primary analysis results on JBrowser, and provide genome browser links for viewing data for other projects, e.g. Gramene/Ensembl Plants browsers. MaizeCODE is supported by an NSF grant IOS 1445025; SciApps is supported by a NSF grant DBI-1265383, and USDA-ARS (1907-21000-030-00D).

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

P13 

## Alternative splicing in the NAM lines using multiple reference genomes.

(submitted by Jerald Noble <[jnoble333@ufl.edu](mailto:jnoble333@ufl.edu)>)

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Maize is a highly polymorphic species exhibiting significant genetic diversity and phenotypic plasticity. The majority of maize genomic and transcriptomic research employs B73 as a reference genome to guide alignments and discovery of relevant characteristics. Alternative splicing (AS) is a process enabling the production of multiple transcript isoforms from the same gene. AS contributes to an increase in proteomic diversity without the need to increase the size of a eukaryotic organism's genome. The dynamics of AS between plant tissues and genotypes in a given species is highly variable with certain AS events being tissue or genotype specific. Here we employ a robust, novel bioinformatics pipeline to discover and compare the AS events between 5 maize tissues in 27 maize lines relative to the B73, W22, PH207, and CML247 reference genomes. Intron retention is the most abundant AS type regardless of the reference genome used. In addition to identifying all of the AS events in these samples relative to these 4 reference genomes, we have assembled reference transcriptomes for the W22, PH207, and CML247 reference genomes using RNA-seq data from these genotypes. Many AS events were discovered that were genotype and tissue specific in the population. Further analysis is needed to provide potential functional insight into the tissue and genotype specific AS events.

Funding acknowledgement: National Science Foundation (NSF)

P14 

## **Augmenting maize image training datasets using simulated photos**

(submitted by Thomas Hoban <[thoban19@comcast.net](mailto:thoban19@comcast.net)>)

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Computer vision and machine learning approaches hold the potential to dramatically change and accelerate plant phenotyping approaches. However, in order to accurately score traits from image data, these approaches require large and well annotated training datasets, which can be both expensive and time consuming to generate. Here we employed an off the shelf software tool – Plant Factory – procedurally generate thousands of 3D models of corn plants. Custom python scripts were then combined with the open source tool Blender to automate the creation of simulated 2D phenotyping images from these 3D models. We evaluated a number of approaches to employing these simulated images to train neural networks to score phenotypes from images of corn plants, and demonstrate that the inclusion of simulated training data can improve the accuracy of neural net-based phenotyping when genuine training data is limiting or completely absent. Ultimately, improved and more biologically informed maize growth models may radically lower barriers in the world of plant phenotyping, as thousands or tens of thousands of simulated images with known ground truth phenotype states can be generated overnight on a conventional desktop computer.

Funding acknowledgement: National Science Foundation (NSF)

P15 

## **Bracing for Impact: The function of aerial roots in maize stability**

(submitted by Erin Sparks <[esparks@udel.edu](mailto:esparks@udel.edu)>)

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Lodging can have a significant impact on cereal crop yield and grain quality. In maize, roots that emerge from stem nodes above the soil, called brace roots, are proposed to play an important role in structural stability and root lodging resistance. Yet there is very little known about these roots. Our research focuses on defining the function of maize brace roots and identifying the molecular regulation of their development. To examine function, we used field-based flex testing devices to show that brace roots do significantly contribute to plant flexural strength. To identify what aspects of brace roots are good for stability, we have analyzed the contribution of brace roots to flexural stiffness in 50 different maize genotypes. We have coupled this analysis with high-throughput field phenotyping and root mechanical testing to determine what brace root features promote stability. Through this analysis, we have identified the brace root traits most advantageous for plant stability, and can leverage this information to identify the genetic regulation of these traits.

Funding acknowledgement: University of Delaware Research Foundation, Thomas Jefferson Fund, Delaware Biosciences Center for Advanced Technology Grant

P16 

## **CGAS: Comparative genetic association studies**

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Genome-wide association studies (GWAS) are a useful resource for finding linked SNPs for any measurable phenotype. However, the results generated from a GWAS tend to be long lists of associated SNPs and candidate genes, many of which are hypothesized to be background noise due to linkage. Many traits have evolved early in the plant lineage, suggesting that the genes driving the traits may be conserved. We have created a comparative genetic association tool (CGAS) to filter through the noise and prioritize GWAS results into a concise list of candidates for follow up studies. CGAS leverages information gathered from multiple GWAS results of different species and orthology to determine which genes are most likely to be associated with a phenotype. Orthology implies evolutionary conserved biological function. Genes linked to significant SNPs for a phenotype in multiple species are more likely to be causative than genes in linkage without orthologs near SNPs in other species. To check for spurious associations, permutations of the original dataset are also tested to ensure the results are more significant than random chance. In preliminary studies, the number of high-confidence, candidate genes returned is inversely related to the number of species used in the comparison. We have found that when using GWAS data from several different phenotypes in at least five species, we identify candidate genes in real data with very few genes identified in permutation sets. Because CGAS does not rely on gene ontologies or annotation, it can be used regardless of a species' level of gene annotation.

Funding acknowledgement: National Science Foundation (NSF)

P17

## **Characterizing the major structural variants among maize NAM genomes with Bionano optical mapping**

(submitted by Jianing Liu <[jl03308@uga.edu](mailto:jl03308@uga.edu)>)

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As the most widely planted crop, maize has adapted to a range of environments. The genome architecture of maize has evolved along with the expansion of its habitat, and extraordinary genetic diversity is present among inbreds. A core set of 25 inbreds known as NAM founder lines were chosen to represent the great diversity of maize. These 25 lines and B73 are currently being sequenced and assembled to enable the comprehensive study of maize genomic diversity. *De novo* assemblies were constructed by incorporating PacBio sequencing, Bionano optical mapping and short read sequencing. Though the soon-to-be released assemblies of NAM genomes will be of exceptional high quality, sequence assemblies over highly repetitive regions can still be erroneous or incomplete. On the other hand, the long-range contiguity of optical maps enables high-confidence assemblies over the complex repeat-rich area. Here we characterize the large-scale structural diversity among 26 maize inbreds with the standalone use of Bionano optical maps. The pipeline involves pseudomolecule construction with *de novo*-assembled DLE maps and comparative genomic analyses. The group-specific and line-specific conserved regions will be identified through whole-genome alignment. Large-scale structural variants will be characterized with Bionano SV detection pipeline and annotated.

Funding acknowledgement: National Science Foundation (NSF)

P18 

## Combinatorial gene regulation of maize phenylpropanoid genes

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Elucidation of gene regulatory networks (GRNs) is one of major areas in plant systems biology, given the intrinsic relationships between phenotypic traits and gene expression profiles. These expression profiles are largely defined by regulatory links between sets of transcription factors (TFs) and their genes targets. In maize, a set of 1,100 protein-DNA interactions (PDIs) between 568 TFs and 54 enzyme genes associated to phenolic metabolism were recently identified<sup>1</sup>. We combined co-expression profiles of the 568 TFs with the entire maize genome, using data from the maize gene expression ATLAS project<sup>2</sup>, and this allowed us to predict new protein-protein interaction and PDIs associated with maize phenolic metabolism. Using this new GRN information, we queried the local or tissue-specific expression of the newly discovered regulatory links. We identified several putative novel protein-protein local-coexpressed interactions and PDIs which are currently being experimentally validated in the lab. The results to be presented highlight the importance of high-quality PDIs and expression datasets to guide predictive biology towards the discovery of novel edges in gene regulatory networks. This research was funded by NSF grant IOS-1733633.

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Funding acknowledgement: National Science Foundation (NSF)

P19 

## Comparative approaches to identify the genetic basis of desiccation tolerance in Poaceae

(submitted by Jeremy Pardo <[pardojer@msu.edu](mailto:pardojer@msu.edu)>)

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Domesticated grasses such as maize, sorghum and rice are the cornerstone of the global food system. However, these crops are often more susceptible to water stress than their wild relatives, with drought being the most prominent abiotic factor limiting crop yield. Some wild grasses are remarkably resilient, to the point of surviving near complete desiccation of their vegetative tissues. Vegetative desiccation tolerance has arisen independently a minimum of nine times within Poaceae, however the genetic factors that differentiate desiccation tolerant species and their desiccation sensitive crop relatives remain largely unknown. Using a comparative genomic, transcriptomic and physiological approach we compared datasets from the crop species maize, sorghum, rice and tef with related desiccation tolerant C4 grasses *Eragrostis nindensis* and *Oropetium thomaeum*, to identify evolutionarily conserved differences. It was previously hypothesized that transcriptional re-wiring of seed desiccation pathways, confers vegetative desiccation tolerance in *E. nindensis* and *O. thomaeum*. We demonstrate that the majority of seed dehydration related genes show similar expression patterns in leaves of desiccation tolerant and sensitive species during dehydration. However, we discovered a small set of orthologs with expression specific to leaves of desiccation tolerant species, and seeds of sensitive species. Thus, our analysis uncovered a set of candidate genes important in conferring vegetative desiccation tolerance in some grasses. These genes represent potential targets for improving the drought resilience of crop plants.

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

P20

## Comparative co-expression gene network analysis reveals evolutionary conservation and divergence of seed shattering mechanism

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Seed shattering, or seed dispersal, is a process by which almost all plants disperse seeds at maturity. Shattering occurs by the formation of an abscission zone (AZ), which is a layer of cells located in various positions between the seed and the parent plant, and is specialized for separation. In grasses, the type of seed shattering varies with the position of AZ formed, and the position is highly variable even in closely related species. In this study, we conducted a comparative co-expression gene network analysis in three grass species with different AZ positions, including weedy rice (*Oryza sativa*), purple false brome (*Brachypodium distachyon*) and green foxtail (*Setaria viridis*), using transcriptome data of AZ and tissues above and below it at two developmental stages. Comparisons between species revealed that network topologies are altered due to the changes in transcriptomic co-expression relationships, particularly in AZ tissue-specific modules. A few modules were also found to be highly preserved among species, but they were only limited to changes between developmental stages, not to differences between the developing abscission zone and the surrounding tissue. These results demonstrate substantial modification of the AZ co-expression gene network during evolution, and provide insights into the differential genetic regulation of seed shattering in grasses.

Funding acknowledgement: National Science Foundation (NSF)

P21

## Comparative transcriptomics to unveil heat stress responsive genes in maize

(submitted by Ashok Jagtap <[ajagtap@purdue.edu](mailto:ajagtap@purdue.edu)>)

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The worldwide production of maize faces serious threat because of heat stress associated with climate change. To be able to develop heat resilient germ plasm, we identified two inbreds LM11 and CML25 that are highly sensitive and tolerant of heat stress, respectively. These inbreds were planted under intense heat in Punjab and samples were taken for transcriptome analyses from three separate tissues (flag leaf, tassel and developing kernels) shortly before leaf firing and tassel blast symptoms developed on the sensitive inbred. RNA-seq library preparation and sequencing were performed using an Illumina HiSeq2500 sequencing machine. A total of 2,164 differentially expressed genes (DEGs) were detected in differential comparisons between LM11 (sensitive) and CML25 (tolerant) samples, with 1151, 451 and 562 DEGs being identified in comparisons of corresponding leaf, pollen and ovule samples, respectively. Functional annotations of DEGs revealed that many DEGs identified corresponded or related to genes already known to be associated with heat stress. This included heat shock transcription factors (HSFs), heat shock proteins (HSP20 & HSP70), as well as genes related to photosynthesis (PsaD & PsaN) and antioxidation (Peroxidases). These results have the potential to enhance our understanding of the maize response to heat stress.



P22 

## Comparing ChIP-seq and DAP-seq binding information using *P1* (*Pericarp color 1*) genome-wide targets as a model

(submitted by Yi-Hsuan Chu <[chuyihsu@msu.edu](mailto:chuyihsu@msu.edu)>)

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DAP-seq is a relatively new and easy *in vitro* method to capture genome-wide transcription factor (TF)-DNA interactions as compared to *in vivo* ChIP-seq assays. The limitations and strengths of each method and how to interpret the results obtained by each in the context of the other have yet to be determined. By using the well-studied maize *P1* gene, which encodes an R2R3-MYB domain protein regulating the flavonoid biosynthesis pathway, we are able to link *in vitro* DNA-binding preferences with *in vivo* DNA-binding complexity and target gene expression. The combination of ChIP-seq and DAP-seq, together with gene expression analysis using contrasting phenotypes provide us a testable biological system to understand the complementarity of DAP-seq and ChIP-seq. In this study, we focus on: 1) The differences and similarities in binding target selection, 2) the peak distribution in maize genomic content, chromatin landscape and DNA structure, and 3) the composition and conservation of identified motifs, and their correlation to the differentially expressed genes. As a result, these analyses can help to unravel the *in vitro-in vivo* relationship, which can lead to a more accurate interpretation of DNA binding from both experimental designs.

Gene / Gene Models described: *p1* - *pericarp color1*; GRMZM2G084799, Zm00001d028854

Funding acknowledgement: National Science Foundation (NSF)

P23

## Culturable leaf and husk microbiome of field-grown maize infected with mycotoxygenic and biocontrol strains of *Aspergillus flavus*

(submitted by An-Anthony Nguyen <[angu124@lsu.edu](mailto:angu124@lsu.edu)>)

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*Aspergillus flavus* is a soil saprophyte and an opportunistic plant pathogen. The fungus infects and contaminates key crops such as maize, peanuts and tree nuts with the most naturally-occurring carcinogenic mycotoxin called aflatoxin B1. The fungus can also directly infect and cause respiratory diseases in immunocompromised patients. Under the supervision of Dr. Chalivendra, I am comparing microbial flora colonizing the leaves and husks of developing ears in two maize in-breds with contrasting resistance to aflatoxin accumulation. B73, a susceptible inbred and CML322, a resistant inbred were grown in LSU Agricultural Center in the summer of 2018. During the mid-silk phase, they were either mock-inoculated or inoculated with a highly toxigenic *A. flavus* strain, a biocontrol strain or their mix. Leaf and husk samples were sampled 5 times during seed development until harvest. I have been focusing on the culturable bacterial and fungal populations that are recoverable on rich synthetic media. Nearly 190 bacterial colonies have been recovered. DNA extraction has already been completed from these bacteria for PCR and sequencing of the 16S rDNA and other amplicons needed for identification. The isolated bacteria and fungi will be tested for their antagonistic effect on *A. Flavus* growth and aflatoxin production *in vitro*. The study will complement the culture-independent approach our group is taking to identify novel, robust and environment-friendly biocontrol organisms to mitigate aflatoxin contamination of our food.



P24

## **Curation of corn RefSeq to provide a robust resource for research and annotation**

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The Reference Sequences (RefSeq) project at NCBI aims to provide a comprehensive set of well-annotated sequence records for a diverse set of organisms. RefSeq records serve as the foundation for NCBI's Gene resource, which provides an integrated view of gene information including gene transcript and protein products, genome annotation, publications demonstrating function of the gene product(s), information about expression, and links to pertinent resources both within NCBI and outside NCBI. Together, RefSeq and Gene provide information for nearly one-thousand eukaryote genomes, including a diverse set of agriculturally-important species.

Curation of corn records by the RefSeq project at NCBI is the effort to create a nonredundant collection of corn RefSeq Genes. Each Gene record contains a nonredundant collection of transcript isoforms, mapping information, publications demonstrating function of the gene product(s), information about expression and links to pertinent resources both within NCBI and outside NCBI.

In the process curation identifies transcribed pseudogenes, identifies corn transcripts that appear to be specific to a cultivar, identifies the precursor transcript for miRNA, identifies examples where translation does not use the 5'-most AUG and identifies instances where the transcript encoding the functional protein should be subject to nonsense-mediated decay. Curation creates corn Gene names in collaboration with MaizeGDB and creates corn RefSeq protein names that can be utilized by the NCBI Eukaryotic Annotation Pipeline to computationally name proteins in other plant species. These data are available through a variety of NCBI resources including BLAST, and via FTP and NCBI's e-utilities APIs.

Funding acknowledgement: National Institutes of Health (NIH)

P25

## **Custom built scanner and computer vision pipeline enables low-cost, large-scale phenotyping of maize ears**

(submitted by Matthew Warman <[warmanma@oregonstate.edu](mailto:warmanma@oregonstate.edu)>)

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Computer vision algorithms are becoming increasingly powerful, dramatically changing our ability to document, measure, and detect phenomena. Unfortunately, taking advantage of these trends can be difficult for scientists with few resources studying unique biological systems. Here, we describe a powerful, cost-effective combination of a custom-built imaging platform and open-source computer vision pipeline. Our maize ear scanner was built with off-the-shelf parts for <\$80. When combined with a cellphone or digital camera, movies of rotating maize ears were digitally flattened into projections covering the entire surface of the ear in one image. Segregating GFP and anthocyanin seed markers were clearly distinguishable in ear projections, allowing manual counting using ImageJ. To speed the counting process, a computer vision pipeline was developed using the scikit-image Python library. Our image collection of >150,000 hand-annotated kernels provides a unique dataset to validate our pipeline. Progress using this dataset to train machine learning kernel identification models is also described. Once annotated, statistically powerful transmission data can be collected for hundreds of maize ears. As a test case, our methods were able to detect small but statistically significant decreases in male transmission rates in mutant alleles of genes shown to be highly abundant in specific developmental stages of the maize male gametophyte. In addition, the imaging methodology allows a detailed assessment of the physical distribution of seed phenotypes on the ear, identifying at least one mutation in which transmission rate varies depending on location relative to the base of the ear.

Funding acknowledgement: National Science Foundation (NSF)

P26

## CyVerse-empowered kernel morphometric analysis of corn hybrids grown in Arkansas

(submitted by Karina Medina Jimenez <[kmedina@astate.edu](mailto:kmedina@astate.edu)>)

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Maize (*Zea mays*) is currently one of the most heavily relied upon crops on earth. Kernel shape is one of the most important morphological parameters related with quality and yield in corn production. Measurements of the geometrical parameters such as area, length, contour length and width have been used to discriminate morphologic variation in seed shape and in the estimation of maize yields. Traditionally, kernels have been measured by manual methods, using calipers, which is a very time consuming and error-prone process that limits the number and the quality of measurements that can be feasibly taken. With the development of high-throughput plant phenotyping, the generation of high quality genotype data for maize is both attainable and advantageous. In this study, using CyVerse cyber-infrastructure together with a imaging processing method developed by ND Miller as part of the Phytomorph pipeline we are able to extract relevant digital readouts such as the area, perimeter, length and width profiles of kernels, as well as color hues from 250 hybrid corn lines grown in Arkansas as part of the Genomes to fields (G2F) project. This process uses digital images acquired with RGB, near infrared (NIR) and fluorescence (FLUO) sensors that are part of a Scanalyzer HTS instrument. Hundreds of thousands of images are being analyzed through the segmentation algorithm that is increasing our knowledge and abilities to evaluate kernel traits in detail and with greater objectivity

P27 

## De novo genome assembly of the 26 NAM founder inbreds

(submitted by Kelly Dawe <[kdawe@uga.edu](mailto:kdawe@uga.edu)>)

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Maize is an important crop and model organism for plant genetics. However, currently, most forms of sequence analysis are referenced to the single B73 inbred. Beyond B73, the most extensively researched maize lines are the core set of 25 inbreds known as the NAM founder lines, which represent a broad cross section of modern maize diversity. Prior data show that gene content can differ by more than 5% across lines and that as much as half of the functional genetic information lies outside of genes in highly variable intergenic spaces. To capture and utilize this variation, the NAM founder inbreds and a twenty-sixth line containing Abnormal chromosome 10 have been sequenced and assembled using PacBio long read sequencing and BioNano optical mapping. RNA-seq data from ten tissues will be used to annotate each genome, and assemblies and annotations will be released with browser support through MaizeGDB and Gramene. Comparative genomic tools are being used to identify and catalog the maize pangenome, and assess the role of structural variation such as presence-absence variation and copy number variation in the determination of agronomic traits. This poster will provide a project overview and updated status of the project.

Funding acknowledgement: National Science Foundation (NSF)

P28

## Differential architectures of hybridization between teosinte subspecies across multiple hybrid zones

(submitted by David Hufnagel <[davidehuf@gmail.com](mailto:davidehuf@gmail.com)>)

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The teosintes *Zea mays* ssp. *parviglumis* and *Zea mays* ssp. *mexicana* are an excellent model system for studying hybridization and hybrid zones as they are diverse, show signs of local adaptation, and readily produce hybrids where their parapatric ranges overlap. We have previously identified three groups of hybrid populations in putative hybrid zones with diverse habitats and genotypes using a broad genome-wide 983-SNP, 2,793-individual dataset. We are now investigating two of our identified putative hybrid zones, along with a third we have not previously explored, using data derived from the maize 55K SNP genotyping platform. This data set provides higher density genotyping and population-level sampling, being comprised of 645 individuals from 49 populations with 33,452 SNPs. The goals of this project are to assess differential genome-wide architectures of hybridization between hybrid groups, to determine whether these could be adaptive, and to predict the age and origins of hybrid groups. Architectures of hybridization may vary due to differential loss and retention of parental haplotypes across hybrid groups following admixture during hybridization. Here, hybrids are modeled as mosaics of parental haplotypes, the lengths of which will be used to explore the timing of hybridization. Differential architectures of hybridization may also be linked to adaptation when particular parental haplotypes are favored in certain environments across individuals.

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P29

## Distinct genotype and tissue-specific TSS usage in B73 and Mo17 maize inbred lines

(submitted by Aimer Gutierrez-Diaz <[gutie190@msu.edu](mailto:gutie190@msu.edu)>)

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Cap analysis of gene expression (CAGE) has been extensively used for the precise identification of capped 5' ends of mRNAs corresponding to the Transcription Start Sites (TSS) of genes. Since this technique was adapted to Next Generation Sequencing technologies, it has provided invaluable information at the genome-wide scale. It has contributed to improving coding-gene annotations and also provides a critical step in the characterization of promoters in terms of shape, tissue- or condition- specific expression patterns. More recently, CAGE has contributed to reporting 5' gene isoforms resulting in the usage of alternate translation start-sites and small ORFs. Here, we extend a previous genome-wide TSSs analysis in root and shoot tissues of two maize (*Zea mays*) inbred lines (B73 and Mo17), using the new available genome version of B73 (AGPv4) and Mo17, which allowed us to assess new questions from a comparative genomics perspective. In a previous study, we identified TSSs for ~17,000 maize genes and discovered an unexpected plasticity in TSS selection<sup>1</sup>. With the development of a comparative genomics pipeline for CAGE analysis, we updated the genome-wide maize TSS map for B73 AGPv4 and Mo17, increasing the quality of the current annotated gene models, because TSS calling is empirically supported. Additionally, for each gene with a CAGE tag signal we provide a promoter characterization based on shape (sharp or broad) and its expression pattern (root-, shoot-specific or general). Furthermore, grouping promoters using the expression levels rather than shape, we recovered a set of promoters with a phylogenetically conserved tissue-specific expression pattern or genotype specific, which permitted us to characterized promoter motifs at this level of analysis, providing some interesting insights with regards to promoter usage in hybrids. Finally, we provide the first catalog of empirically supported gene 5' isoforms driven by alternative TSS usage, which may impact the maize proteome, by molecular mechanisms including different ATG usage and exon exclusion.

Funding acknowledgement: National Science Foundation (NSF)

P30

### **DNS-seq maps of nucleosome occupancy and open chromatin in B73 root tip, coleoptilar nodes, earshoot, and 15-DAP endosperm.**

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The goal of this project (referred to as NUPRIME) is to develop micrococcal nuclease (MNase) profiling as a foundational resource for integration of maize epigenomic data. The web page at [maizenucleosome.org](http://maizenucleosome.org) describes the project, supported by the Plant Genome Research Program (NSF IOS 1444532). We previously showed that particular genomic regions are highly susceptible to variation introduced by differences in the extent to which chromatin is digested with MNase (Vera et al., 2014). We exploited this digestion-linked variation to simultaneously map nucleosome occupancy and open chromatin while defining the functional portion of the maize genome (Rodgers-Melnick et al., 2016). The open chromatin is nuclease hypersensitive and operationally defined by differential nuclease sensitivity (DNS). Here we present DNS-seq chromatin profiles on four distinct maize B73 tissues; root tip, coleoptilar nodes, earshoot, and mid-maturation endosperm. We also developed and applied a new peak-calling algorithm, iSeg (Girimurugan et al. 2018), to identify discrete sites of open chromatin and facilitate comparative genomics. These data along with a wet-bench protocol and data processing pipelines are published in Turpin et al., 2018 (DOI:10.1016/j.dib.2018.08.015) and accessible via the UCSC maize genome browser at [genomaize.org](http://genomaize.org). Comparative analyses between tissues are currently underway.

Funding acknowledgement: National Science Foundation (NSF)

P31

### **Dynamic changes of root proteome reveal diverse responsive proteins in maize subjected to cadmium stress**

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Toxic symptoms and tolerance mechanisms of heavy metal in maize are well documented. However, limited information is available regarding the changes in the proteome of maize seedling roots in response to cadmium (Cd) stress. Here, we employed an iTRAQ-based quantitative proteomic approach to characterize the dynamic alterations in the root proteome during early developmental in maize seedling. We conducted our proteomic experiments in three-day seedling subjected to Cd stress, using roots in four time points. We identified a total of 733, 307, 499, and 576 differentially abundant proteins after 12, 24, 48, or 72 h of treatment, respectively. These proteins displayed different functions, such as ribosomal synthesis, reactive oxygen species homeostasis, cell wall organization, cellular metabolism, and carbohydrate and energy metabolism. Of the 166 and 177 proteins with higher and lower abundance identified in at least two time points, 14 were common for three time points. We selected nine proteins to verify their expression using quantitative real-time PCR. Proteins involved in the ribosome pathway were especially responsive to Cd stress. Functional characterization of the proteins and the pathways identified in this study could help our understanding of the complicated molecular mechanism involved in Cd stress responses and create a list of candidate gene responsible for Cd tolerance in maize seedling roots.

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P32

## Estimation of the divergence between maize and sorghum using the Andropogoneae

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The identification of regions showing evolutionary conservation between two species is limited by the breadth of experimental manipulation or surveys of existing variation between species. Such variation that exists between species is often confounded by deep coalescence events and divergent physiology. A more relevant sample of independent evolution spanning one billion years can be obtained by sequencing ~900 grasses in the Andropogoneae clade. All of these grasses exhibit NADP-ME C4 photosynthesis and are thought to share a common ancestor that existed between 16 and 20 million years ago. Here we outline our study using assembled Andropogoneae genomes to estimate evolutionary rates between maize and sorghum applying various methods to estimate base conservation. Genomic Evolutionary Rate Profiling (GERP) is one method of estimating constraint from sequence alignments. GERP estimates will be compared between three groups: solely within the Andropogoneae, within the Poaceae, and with available angiosperm genomes. By performing this analysis over broad evolutionary time scales, patterns of evolutionary constraint can be obtained. This information could help to infer a more accurate picture of the divergence between maize and sorghum and help to inform plant breeders on opportunities for heterosis effects and loci for gene editing.

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

P33 

## Expression analysis in maize roots describes gene regulatory relationships and informs on previously mapped leaf and seed ionome QTL

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Roots of young plants undergo highly regulated gene expression changes that pattern root architecture and physiology, with lifelong effects on the structural integrity, water-use efficiency, and nutrient flow of the plant. Many phenotypes, such as seed and leaf element accumulation are often determined by gene expression in the root. To understand gene regulatory networks in maize roots, we measured transcript levels in two-week-old roots of 218 greenhouse-grown plants belonging to the maize Intermated B73 x Mo17 (IBM) recombinant inbred population. We also profiled the ionome of leaf samples from the same plants and carried out QTL mapping on 20 element traits. After performing quality control on the root RNA-seq data, we retained an average of 19.6 million reads per sample. Following quantification with an alignment bias-reducing pipeline, gene expression estimates were used for expression QTL (eQTL) mapping and co-expression analysis which identified 12,497 cis-eQTL, 6,128 trans-eQTL, and 250 co-expressed gene clusters. We detected 8 trans-eQTL hotspots, and found significantly enriched co-expression and gene ontology among hotspot gene targets. Finally, we performed a correlation analysis between root gene expression and leaf element measurements. For 10 elements, genes where root expression correlated with leaf element content co-located with leaf QTL mapped for the element. Additionally, for cadmium and zinc, correlated genes on different chromosomes had trans-eQTL mapping back to the element QTL. The chromosome 2 locus associated with both leaf and seed cadmium content co-localizes with the trans-eQTL hotspot on chromosome 2, which has among its gene targets the top 5 cadmium-correlated genes outside of the QTL interval. Dissecting these relationships can aid in understanding mechanisms and candidate genes underlying element accumulation QTL detected in the leaf and seed.

Funding acknowledgement: United States Department of Agriculture (USDA)



P34 

### Gene sequence classification using k-mer Naïve Bayes model in maize

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Machine learning and modeling approaches have been used to classify protein sequences for a wide set of tasks including predicting protein function, structure, expression, and localization. We describe two k-mer Naïve Bayes classifiers (NB(k) and tNB(k)) for sequence classification. NB(k) constructs, for each class, a k-1 order Markov model, a modified Naïve Bayes model to explicitly model the dependencies (of order k-1) between the letters of a sequence. The k-grams are constructed based on the 4 letters for DNA sequence and 20 letters for amino acid sequence, and it only considers relative frequencies of each respective alphabet the under Naïve Bayes classifier. It may not represent the complexity of protein structure, and it is necessary to introduce more efficient/effective way to extract features from gene sequence. We used k-mer method to pick a random variable with longer sub-sequences and built a k-mer dictionary for each tissue group to support the complexity of protein structure. We extended NB(k) to two-phase Naïve Bayes classifier called tNB(k). To demonstrate the utility of these classifiers, we constructed classifiers to predict gene expression and protein abundance in 23 different maize tissues. In the first phase, we built a feature vector using the predictions from NB(k) with each selected tissues, and then the feature vector was used for final classification in second phase using Bayesian Networks and Deep learning. Our experimental result shows that tNB(k) can achieve higher classification accuracy compare to the traditional Naïve Bayes classifier.

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P35

### Genome-Phenome Eide Association Study (GPWAS): Using high dimensional phenotype data to identify the the genes that specify the traits of maize

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Diverse computational biological tools were developed to provide evidences on supporting *in silico* annotated genes, however, only a small percentage of genes possess validated functional roles. GWAS attempts to link phenotypic variation and genetic variants across the genome. Currently, the number of plant phenotypes which have been characterized in the same association populations, and the number of environments in which those populations have been grown and phenotyped is rapidly growing. High throughput phenotyping technology will accelerate this trend, as it will become possible to measure many more distinct plant traits from the same set of raw plant sensor data. The expanded phenotypic datasets enable another method to test associations between specific genes and variation in target traits. Here we propose the Genome-Phenome Wide Association Study (GPWAS) model, which operates on the principle of reversing the relationship in the variables of a conventional GWAS. GPWAS simultaneously selects an optimum combination of phenotypes to explain maximum variants of a single gene in a population level and uses permutation testing to evaluate the statistical significance of this association. Using whole genome resequencing data from Maize HapMap3 with a set of simulated phenotypes, we demonstrate that GPWAS model can provide additional power relative to FDR when analyzing large sets of phenotypic data include many traits with low heritability. Using genotype and phenotype data of maize retrieved from Panzea, GPWAS detected genes were found to enrich many more GO terms and with greater statistical significance than genes detected by conventional GWAS models. Genes displayed by GPWAS tend are more likely to be independently validated than conventional GWAS models using the same dataset. In summary, these results suggest that genes identified by GPWAS are more likely to have plant functionally constrained roles in determining phenotype.

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## Genome-wide nucleotide patterns and the potential role of UV induced mutation following maize domestication

(submitted by Jianming Yu <[jmyu@iastate.edu](mailto:jmyu@iastate.edu)>)

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Domestication provides a unique model to study genome evolution. Many studies have been conducted to examine genes, genetic diversity, genome structure, and epigenome changes associated with domestication. Interestingly, domesticated accessions have significantly higher [A] and [T] values across genome-wide polymorphic sites than accessions sampled from the corresponding progenitor species, and this pattern was found in multiple comparisons of accessions separated by a population bottleneck. However, the relative contributions of different genomic regions to this genome divergence pattern and underlying mechanisms have not been well characterized. Here we show that non-genic part of the genome has a greater contribution than genic SNPs to the [AT]-increase observed between wild and domesticated accessions in maize. The separation between wild and domesticated accessions in [AT] values is significantly enlarged in pericentromeric regions. Moreover, motif frequency and sequence context analyses showed the motifs (PyCG) related to solar-UV signature (5'-Py-mCG-3') are enriched in pericentromeric and nongenic regions, particularly when they are methylated. Additional analysis using population-private SNPs also implicated the role of motifs related to solar-UV signature in relatively recent mutations. With base-composition across polymorphic sites as a genome phenotype, genome scans identified a set of putative candidate genes involved in UV damage repair pathways. Interestingly, similar patterns were observed in soybean and are expected for other species if large-scale resequencing is completed for both wild and domesticated accessions. Our findings establish the important links among UV radiation, mutation, DNA repair, methylation, and genome evolution. For example, we may ask the following questions: *Is the frequent transition of methylated C to T actually a cost that genomes have to pay for having transposons and repetitive sequences methylated? How far will this AT-biased genome divergence continue? Should future mutation accumulation experiments in model species be carried out with contrasting starting materials and under different mutagen treatment regimens?*

Funding acknowledgement: National Science Foundation (NSF)

P37

## GenomeQC: An R/Shiny-based quality assessment tool for plant genome assemblies and gene annotations

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Genome assemblies are foundational for understanding species' biology. They provide a physical framework for mapping DNA and RNA sequence, and thus enable characterization of nucleotide diversity, gene expression and prediction across individuals and species. To enable informed use of genome assemblies for these applications, it is important to assess and report assembly quality. Without quality metrics, it is possible to make incorrect assumptions regarding the completeness and contiguity of an assembly, leading to incorrect conclusions. Currently, the quality of a newly sequenced genome is assessed using a set of commonly calculated metrics that are then compared to gold standard reference genomes. While there exists several tools for individual metrics, for both genome and annotation, applications integrating several of these tools together to provide comprehensive evaluations, are surprisingly non-existent. Here, we describe a new genome assembly quality toolkit that integrates multiple metrics to characterize assembly and gene annotation quality.

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P38

## Haplotyping using full-length transcript sequencing in maize F1 hybrid reveals allele-specific expression

(submitted by Bo Wang <[bwang@cshl.edu](mailto:bwang@cshl.edu)>)

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Maize is an important genetic model for elucidating transcriptional networks. Haplotype phasing of genetic variants is important for interpretation of the maize genome, population genetic analysis and functional genomic analysis of allelic activity. Recently, full-length transcript sequencing using long read technology has enabled us to characterize alternative splicing events and improve the maize genome annotation. However, the general Iso-Seq algorithm ignores SNP-level information, focusing instead on identifying alternative splicing differences. Here, we present an algorithm called IsoPhase that post-processes Iso-Seq data for transcript-based haplotyping. For each gene, IsoPhase gathers the associated full-length reads, each representing a single transcribed molecule. It then calls SNPs and is able to infer the haplotype of the reads due to the full-length nature of the sequencing. We applied IsoPhase to a maize Iso-Seq dataset consisting of two homozygous parents (B73 and Ki11) and two F1 reciprocal hybrids (B73xKi11, Ki11xB73). We validated the majority of the SNPs called with IsoPhase against matching short read data and identified cases of allele-specific, gene-level and isoform-level expression. Our results show that maize parental lines and hybrid lines display different splicing activity, and 6,847 genes can be phased through IsoPhase in two reciprocal hybrids using embryo, endosperm and root tissues. Our study identified parental origin isoforms in maize hybrids, different novel isoforms between maize parent and hybrid lines, provides measures of haplotypic expression that increase power and accuracy in studies of allelic expression. It is the first study of phased full-length isoforms in maize, as well as in plants, which provides insights about maize and plant heterosis at allele-specific full-length transcriptional level. The approach used in this study also provide important information for many other phasing studies in different species.

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P39 

## High-throughput mapping of the *in-vivo* binding of 104 maize transcription factors using ChIP-Seq

(submitted by Xiaoyu Tu <[tuxiaoyuerin@gmail.com](mailto:tuxiaoyuerin@gmail.com)>)

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Multiple GWAS studies have shown that important genetic variation resides in the non-coding regions of the maize genome. However, the mechanistic knowledge that would allow to link “trans” activity to “cis” mutations resides in the gene regulatory network, which remains poorly characterized besides scattered efforts. We sought to systematically catalog the binding of transcription factors (TFs) in the maize leaf. To achieve this, we have developed an efficient leaf protoplast transformation system and a new hyperstable transposase (HS-Tn5) for ChIP-Seq. In brief, we first removed the water-impervious leaf lower epidermis to expose the inner cells to cell wall digestion enzymes, which resulted in the gentle release of intact protoplasts, ideal for PEG-mediated transformation with high efficiency (>90%). The expressed TFs was fused to biotin epitope tag (3xAviTag). The strong biotin-streptavidin binding permits the use of stringent washes that can suppress the background noise. Ultra low input ChIP-Seq library can then be constructed using HS-Tn5. Using this system we have performed ChIP-Seq for 104 TFs with biological replicates. We identified 2,162,941 highly-reproducible TF binding peaks (Irreproducible Discovery Rate 1%), most of them (~57%) located in gene proximal regions ( $\pm 2.5$  kb from the TSSs). To complement the TF-centric approach we performed ATAC-seq for maize leaf mesophyll cells, and bundle sheath cells. About 99% of the active chromatin regions are overlapped with TF-binding peaks, which suggests that our TF collection is large enough to inform about the gene regulatory mechanism operating in the maize leaf. To the best of our knowledge, it is the largest map of *in-vivo* regulatory regions in a given plant.

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## Highly genotype- and tissue-specific single-parent expression drives dynamic gene expression complementation in maize hybrids

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Maize exhibits tremendous diversities for gene expression and the combination of this diversity could have important impacts on the manifestation of heterosis in hybrids. In this study, we used transcriptome data for five different tissues from 33 maize inbreds and 89 hybrids (430 samples in total) to survey the transcriptomic landscape of the F1-hybrids relative to their inbred parents across diverse genotypes and tissues. Analysis of this data set revealed that single parent expression, while commonly observed, is highly genotype- and tissue-specific. Genotype-specific single parent expression genes also tend to be tissue-specific, and vice versa. Genes with single parent expression caused by genomic presence/absence variation (ePAV) have a much higher rate of expression of the presence allele in hybrids compared with expression of genes caused by gene silencing in one of the parental lines (SPE) (74% vs. 55%), which may indicate different roles in the manifestation of heterosis. For SPE genes, allele specific expression was used to investigate if parental alleles that are not expressed in the inbred line (“silent allele”) get reactivated in the hybrid. We found the reactivation of the silenced allele in hybrid is relatively rare (~7.3% of SPE genes), but is observed across almost all hybrids and tissues. Finally, SPE and ePAV genes are highly overrepresented for being non-syntenic while underrepresented for genes with evidence for possible phenotypic roles. This study provides a more comprehensive understanding of potential role of SPE and ePAV genes in heterosis from the perspective of the transcriptome.

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P41 

## Hybrid maize in the genomics era: single parent expression, complementation and heterosis

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Reference quality assemblies are becoming more common with dropping costs of sequencing and computational resources. The new, *de novo*, long-read assemblies of the founders of the maize Nested Association Mapping (NAM) population open up a number of possible comparative genomic analyses. These maize lines can be compared directly to each other or be used as references, utilizing previously generated re-sequencing data. This could be particularly useful in understanding expression patterns related to hybridization. For instance, researchers have found genes that are expressed in one inbred maize parent and its F1 hybrid offspring, but not in the second parent. This pattern is called single parent expression and has relevance, for example, to complementation models of heterosis. However, the mechanism underlying this phenomenon is not well understood. Here we utilize the NAM founder assemblies to assess variation at previously identified SPE genes. We investigate how structural variation, such as presence-absence variation (PAV), may correlate with observed SPE patterns. We then discuss our current efforts to expand the known set of SPE genes through creation of a diallel using a subset of NAM founders. We will use this new resource to clarify the extent to which SPE is driven by PAV versus regulatory variation through analysis of open chromatin based on ATAC-seq datasets for each NAM line. As we hone our understanding of the governing mechanism of SPE patterns, this may help explain heterosis and other aspects of hybridization.

Funding acknowledgement: National Science Foundation (NSF)

P42

## Identification of abiotic stress responsive genes in *Zea mays* (maize) dependent on MOP1-mediated epigenetic regulation and the plant hormone ABA

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*Zea mays* (maize, corn) is an essential crop plant; important to global agriculture and the U.S. economy. However, maize productivity and yield can be drastically affected by abiotic environmental stress. Therefore, a priority for many plant breeding programs is to select for crops displaying phenotypic traits of enhanced tolerance to abiotic stress. A subset of abiotic stresses induce the plant hormone, abscisic acid (ABA). The mediator of paramutation1 (*mop1*) gene encodes an RNA-dependent RNA polymerase that functions in the RNA-directed DNA methylation (RdDM) pathway. The *mop1-1* mutation results in the loss of DNA methylation which in turn causes a variety of genes to be expressed abnormally. We will determine how a mutation in a *mop1-1* affects RNA expression under abiotic stress by conducting a computational analysis of multiple RNA-seq datasets of stress-treated maize seedlings. We are comparing RNA-seq data from *mop1-1* and WT seedlings treated with exogenous ABA control (no ABA treatment, Vendramin et al.) with a publicly available dataset of WT maize plants treated with heat, cold, drought, salinity, and control (no stress treatment, Li, P. et al. 2017). Genes commonly down-regulated in the four stresses and in MOP1 WT ABA, but up-regulated in *mop1-1* ABA will represent genes potentially silenced under stress that require MOP1 for gene silencing. The presence of these genes in the given stress treatment allows us to identify the abiotic stress responsive genes that require ABA and MOP1 mediated regulation.

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## Identification of new genes involved in C4 photosynthesis using comparative genomics and big data

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C4 photosynthesis has repeatedly evolved from the ancestral C3 state, contributing a quarter of the primary productivity on earth. The biochemical reactions of the C4 cycle are separated between M (mesophyll) and BS (bundle sheath) cells, and the expression of C4 genes are regulated in a M or BS specific pattern. Transcriptome and epigenetic (Bisulfite-Seq, ATAC-Seq, histone 3 modification) datasets of three C4 species (maize, sorghum, foxtail millet) for M and BS cells were generated to better understand the function of C4 photosynthesis and to identify candidate genes associated with C4 pathway. One hundred random forest machine learning classifiers were established by integrating 208 expression and epigenetic features in maize, using the known 33 C4 genes and randomly selected 33 negative genes as training data. The features used in the classifiers can be divided into four categories, including chromatin accessibility, DNA methylation, histone 3 modifications, and transcriptional activity. Seven hundred and fifteen genes have features that are similar to the benchmark C4 genes by applying the models genome-wide and they may be part of the C4 photosynthetic genes. Using the same method, 852 and 610 candidate genes were found in sorghum and foxtail millet, respectively. A total of 195 syntenic genes overlap in these 3 species, which are mainly enriched in photosynthesis and carbohydrate metabolism related GO terms. Transcriptional activity features are the most important kind of features with the AUC value that is almost similar to the full model. At the same time, several most confident candidate genes were selected for CRISPR-Cas9 knock out in setaria.

Gene / Gene Models described: *NADP-ME*, *PPDK*; Zm00001d000316, Zm00001d038163

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## Implementation of a multi-omics approach to identify highly correlated signatures in maize drought stressed nodal roots

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The development of high throughput techniques in the field of genomics, transcriptomics, proteomics and metabolomics has led to an increasing number of high-quality datasets. In recent times, due to the decreasing costs of such techniques, researchers are able to conduct multiple omics analysis under the aegis of one project. Omics datasets such as transcriptome, proteome and metabolome are being generated from the same sample, giving us a snapshot of all the processes taking place at the same time, in the same condition. Traditional biological data analysis relied on identifying a subset of interesting molecular signatures in each dataset, and then looking at associated signatures in the next omics dataset, essentially in a funnel down approach. A multiomics analysis combines all these omics datasets and more complex data analysis methods are used in a holistic manner to look at all the signatures together. This usually leads to finding previously unknown connections between molecular signatures. Combined with the multiple genotypes and time points, this gives the opportunity to generate highly accurate understanding which can then be used to develop models for translational research.

We showcase the implementation of a high throughput multiomics pipeline which analyzes combined transcriptome, proteome and metabolome data generated from samples of field grown and lab grown drought stressed maize nodal roots. This data has been generated from two inbred maize lines, B73 and drought resistant FR697. The pipeline finds highly correlated groups of elements spanning the different ‘omics’ levels with reference to well-watered, severe stressed and medium stressed root elongation profiles of the node 2 roots from the maize lines.

Funding acknowledgement: National Science Foundation (NSF)

P45

## Inferring maize auxin gene regulatory networks through graph neural networks

(submitted by Juexin Wang <[wangjue@missouri.edu](mailto:wangjue@missouri.edu)>)

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Gene regulatory networks provide useful biological insights into factors affecting the genes’ behavior in a complex biological system. Some techniques exist for inferring regulation using a combination of Chip-Seq and transcriptome datasets, or separately analyzing transcription factors and other gene expression data to identify patterns. However, due to lack of such a combination of data availability, efficiently inferring the gene regulatory networks is a far from a solved problem in bioinformatics. In this work, we introduce an unsupervised framework that explicitly infers rational gene relationships from gene expression data. A graph neural network based variational auto-encoder model is proposed to learn the latent codes, which present the gene relationships as underlying edges of the gene regulatory networks. In the training process, data augmentation is implemented to feed the model with sufficient samples. Our model demonstrates comparable performances on gold standard benchmarks of gene regulatory network construction problems. We also applied our model on maize auxin RNA-Seq data, and part of the inferred regulation relationships between maize auxin response factors and their targets are confirmed by DAP-Seq.

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

P46

### **iRNA-sequencing analysis determines transcriptional activity in *Zea mays***

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In analyzing gene expression, the most widely used approaches assay for changes in transcript abundance. Although useful information, this type of analysis does not provide specific information about the relative contributions of transcriptional and post-transcriptional regulation of gene expression. We have modified a computational method called intron RNA-sequencing (iRNA-seq) analysis to determine genome-wide transcriptional activity in plants using total RNA-seq datasets. iRNA-seq analysis assesses intron coverage and mature transcript levels to identify changes in gene expression that are predicted to result from changes in transcriptional activity. We have used this technique to evaluate transcriptional activity genome-wide in different tissues and genotypes of maize. Using these results, we can predict the relative contribution of transcriptional changes to important plant developmental and regulatory events. This approach is an effective way to improve our understanding of transcriptional regulation, gene silencing, and Pol II activity in plants.

Funding acknowledgement: National Science Foundation (NSF)

P47

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

(submitted by Shuai Zeng <[zengs@mail.missouri.edu](mailto:zengs@mail.missouri.edu)>)

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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. 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 500 registered users. Users working on other biological organisms including plants, animals and biomedical diseases have similar needs and the developed framework can be expanded to make the visualization and analysis tools function for other organisms, without having to reinvent the wheel. To achieve this we have developed KBCCommons, 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 incorporates several multi-omics datasets including transcriptomics, proteomics, metabolomics, epigenomics, molecular breeding and other types. We have currently expanded KBCCommons framework and tools to *Zea mays*, *Arabidopsis*, *Mus musculus* and *Homo sapiens*. We have integrated various genomics dataset for maize including RNAseq B73 mutants and Tassel meristem from our collaborators. It provides a suite of tools such as the gene/metabolite pathway viewer, Protein Bio-Viewer, heatmaps, scatter plots and hierarchical clustering. It also provides access to PGen, Pegasus analytics workflows developed for genomics variations analysis. It also has suite of tools for differential expression analysis of transcriptomics and other multi-omics datasets including venn diagrams, volcano plots, function enrichment and gene modules.

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



P48

## Linking gene expression to nitrogen use efficiency via integrative phenotypic, transcriptomic, and machine learning analyses

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The application of synthetic nitrogen fertilizer to farmland has stimulated crop yield in the past fifty years yet by now reached a plateau. Further increases in applied nitrogen may lead to more environmental issues than higher crop productivity. To the end of simultaneous improved nitrogen use efficiency and high yield, we have exploited genetic diversity and grown, under controlled nitrogen environment, eighteen *Arabidopsis* accessions in the laboratory and seventeen maize genotypes in the field. As might be expected, we find that nitrogen level (N) is the major driver explaining the variation in phenotypes and gene expression levels, followed by genetic background (G) and the G by N interaction. Using the matched phenotypic and transcriptomic data in *Arabidopsis* and maize, we comparatively characterize the differentially expressed genes and identify N-regulatory genes conserved intra- and inter-species. Interestingly, these genes are predictive of nitrogen use efficiency through the utilization of a gradient boosting machine learning tool, XGBoost. We selected some of these novel genes with a feature important value calculated by XGBoost algorithm and observed phenotypes in nitrogen use efficiency using loss-of-function mutants. Using this experimental design and analysis pipeline, we present an approach that links the gene expression levels to a physiological trait of interest and can be applied to any other organisms.

Funding acknowledgement: National Science Foundation (NSF)

P49

## Mapping cis-regulatory variations in plant genomes

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Binding of transcription factor (TF) protein to specific locations in the genome is a key step in the regulation of gene expression. While DNA sequences are a major determinant of TF binding, recent studies showed that the epigenome, including chemical modifications to DNA such as methylation and the associated histone proteins, have a major impact on the binding of TFs to their binding sites. A substantial portion of the intraspecific genetic and epigenomic variations that are linked to phenotypic variations (e.g., plant traits) are in non-coding regions and have been postulated to affect TF binding. We have previously reported the creation of DAP-seq (DNA affinity purification sequencing), a method that couples affinity-purified TFs with next-generation sequencing of a genomic DNA library for rapid generation of genome-wide maps of TF binding sites in native genomic context with endogenous methylation patterns. Using DAP-seq we successfully resolved sequence motifs and binding sites for 529 *Arabidopsis thaliana* TFs at \$40 per TF, annotating 9.3% of the *Arabidopsis* genome as potential cis-regulatory elements. Importantly, DAP-seq interrogates TF binding on DNA that retains its endogenous 5-methylcytosine patterns, allowing us to determine that over 75% of the TFs surveyed are sensitive to 5-methylcytosine in their binding sites. Here we describe the development of experimental approaches and computational framework to enable the application of DAP-seq to map TF binding variations in population genomes with sequence and methylation differences. Using *Arabidopsis* accession genomes in proof-of-principle experiments, we demonstrate the utility of the method in elucidating conserved and population-specific gene regulatory networks as well as in providing insights into the functions of genome and methylome variations.

Funding acknowledgement: National Science Foundation (NSF)

P50 

## Mapping the maize cistrome using MOA-seq (MNase Open & Accessible): Motif discovery and annotation.

(submitted by Hank Bass <[bass@bio.fsu.edu](mailto:bass@bio.fsu.edu)>)

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Chromatin structure is dynamic and intimately linked to gene regulation and other genetic functions. Our lab has developed a nuclease sensitivity profiling method (DNS-seq, [Turpin et al., 2018](#)) that reveals sites of open chromatin linked to biological traits such as growth and yield. Here we set out to develop a new MNase-based open and accessible (MOA) chromatin mapping technique based on isolation and sequencing of small, sub-nucleosomal-sized fragments from light nuclease digests of chromatin from formaldehyde-fixed nuclei. Experimental advantages of MOA-seq include the small amount of tissue required (< ~ 1g), the ability to use frozen or fixed tissues allowing for flexibility in harvest scheduling, and the relatively small number of sequence reads required (60M reads combined / tissue sample). Size selected libraries (~50-130 bp genomic fragments) were sequenced and aligned to the genome. The MOA-seq peaks aligned with DNS-seq hypersensitive footprints, but at higher bp resolution. Read coverage profiles were converted to peak segments using iSeg ([Girimurugan et al., 2018](#)). Motif analysis of the top 1% of the iSeg MOA-seq peaks resulted in the identification of hundreds of motif families. MOA-seq motifs were annotated according to their location relative to genes, their tendency to overlap fragment mid-points, their relationship to open chromatin mapped by other methods (DNase, ATAC), matches to DAP-seq sequences, or similarity to previously mapped ChIP-seq peaks in maize or other plants. Overall, we find that MOA-seq captures a broad array of small-particle footprints genome wide, which we interpret as occupied cis-acting elements (the earshoot cistrome). This innovation adds to the methods for identifying regulatory regions, contributing to the encyclopedia of DNA elements and chromatin structural features in maize. This work was supported by the Plant Genome Research Program (NSF IOS 1444532).

Funding acknowledgement: National Science Foundation (NSF)

P51 

## Match seq: developing a tool for aligning and visualizing ChIP seq, DAP seq, and RNA Seq

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RNA Sequencing (RNA Seq), Chromatin Immunoprecipitation sequencing (ChIP Seq), and DNA Affinity Purified sequencing (DAP Seq) are all modern forms of high throughput sequencing for determining gene expressions and transcription factors binding and regulation respectively. All three are forms of next generation sequencing and help determine gene regulatory information. The next generation sequencing reads are aligned to reference and cuff-diff, edge R or MACS software are used to determine differential expression. There is not however, an easy tool for aligning and searching the RNA Seq data with corresponding ChIP Seq or DAP Seq data.

Using the KBCCommons database and the maize reference genome I have developed a tool for searching and aligning genes with their respective regulating transcription factors. A combination of PHP, Javascript, HTML, Python, JSON, AJAX, and MySQL were utilized to create and implement this web-based tool. Given data previously uploaded to the database, and a(n) transcription factor(s) the tool will present the peaks for transcription factor and respective genes downstream of them based on chromosome coordinates. It will display several statistical measures of the confidence behind the relation, as well as location and heights of ChIP seq or DAP seq peaks. In the future we hope to add additional comparative functionality between the data sets, the ability to add several RNA Seq data sets as well as specify families of transcription factors to search for.

Funding acknowledgement: National Science Foundation (NSF)

P52

## Metabolome and transcriptome responses to a water deficit time course in the model C4 grass *Setaria viridis*

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As a first step in understanding the conserved gene networks underlying the metabolic response to drought in biofuel relevant C4 grass species, we used untargeted metabolomics and transcriptomics to profile *Setaria viridis* at multiple time points during drought stress. We quantified the levels of 7,486 metabolites using hydrophilic interaction chromatography (HILIC) and another 6,157 metabolites using reversed phase liquid chromatography (RPLC), along with 35,065 transcripts using 3' end labeled RNA seq. This will allow us to identify key pathways impacted by drought, and describes how their behavior shifts across time. We selected drought responsive metabolites and used principal component analysis (PCA) as well as partial least squares discriminant analysis (PLS-DA) to detect a peak in the drought response at day 6 across the metabolome and transcriptome. We are in the process of conducting the same analysis in *Sorghum bicolor*. Using *Setaria viridis* and *Sorghum bicolor* diversity panels we will conduct a dual species metabolomic GWAS of drought stress to explore the role of conserved genes regions in regulating the response to water deficit in both species.

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P53

## Metabolome-transcriptome integration by multivariate analysis to dissect the cuticular lipid biosynthesis network on maize silks

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The plant cuticle is infused with non-polar and amphipathic lipids that form a hydrophobic protective layer against environmental stresses. Maize silks are rich in cuticular hydrocarbons that are produced within the epidermis via the reduction of very-long-chain fatty acid (VLCFA) precursors and subsequent decarbonylation of resulting aldehyde intermediates. This sequence of reactions occurs with VLCFA precursors of different chain-lengths, thereby generating a homologous series of VLCFAs, aldehydes, and hydrocarbons that comprise the cuticular lipid metabolome. To understand the dynamics of cuticular hydrocarbon biosynthesis under various conditions, we queried the cuticular lipid metabolomes and transcriptomes of silks from four genotypes (B73, Mo17 and their reciprocal hybrids) sectioned into five segments along the silk length that capture acropetal development and the environmental transition as silks emerge from the husks. Six out of seven pairs of VLCFA precursors and hydrocarbon products were identified by partial-least-square (PLS) discriminant analysis as contributing predominantly in distinguishing between genotypes or among silk sections. Moreover, product-precursor ratios are dynamic along the silk length, among genotypes, and even across precursor carbon chain-lengths. To explore the metabolite-transcript associations impacting this pathway, a PLS-regression model, which is effective in dissecting unbalanced high-dimensional omics data with small sample sizes, was built with product-precursor ratios at different chain-lengths as response variables and transcripts as explanatory variables. Of the ~200 transcripts associated with product-precursor ratios, many are involved in key steps of hydrocarbon synthesis and accumulation, including de novo VLCFA elongation, aldehyde decarbonylation and extracellular lipid transport. In addition, PLS-regression using the transcriptome and concentrations of individual metabolites identified both conserved and unique metabolome-transcriptome associations across chain-length-based homologous hydrocarbon-producing networks. Further analysis is in progress to generate a network that integrates metabolomes with correlation-based gene clusters and will provide deeper insights into the cuticular lipid biosynthesis network.

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



## Mining maize with gramene

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Need to find orthologs for your favorite maize genes? How about expression? Would obtaining a sorghum mutant of your gene of interest accelerate your research? Or perhaps you need to compare entire pathways, and visualize the maize interactome? Gramene (<http://www.gramene.org>) is an integrated resource for comparative functional analysis in plants. We provide access to 57 reference genomes, and pathways for 78 plants species. Built upon Ensembl, Reactome, and Expression Atlas infrastructures, Gramene is committed to open access and reproducible science based on the FAIR data principles. Gramene provides integrated search capabilities and interactive views to visualize gene features, gene neighborhoods, phylogenetic trees, expression profiles, pathways, and cross-references. Maize reference genomic data include the B73 RefGen\_V4 assembly with i) functional descriptions for ~30K genes, ii) sub-genome designation and ohnologs, iii) annotated transposable elements, iv) methylation data, iv) V3-V4 gene ID mappings and an assembly converter to lift-over genomic coordinates between V2, V3, and V4. Gramene hosts genetic variation for 12 species including the Panzea 2.7 GBS and HapMap2 datasets in maize, and the USDA-ARS sorghum EMS collection. The Plant Reactome hosts 280 reference pathways curated in rice and projected to maize and other species by orthology. Visualizations of EBI Expression Atlas data, from almost 800 experiments, are integrated into the search results panel, and both the genome and pathway browsers. Finally, in collaboration with the genome assembly of NAM founders project (NSF IOS-1445025, PI: K. Dawe, Co-PIs: M. Hufford & D. Ware), we are developing new resources to interrogate the maize pangenome. Now in development, the Gramene Maize Pangenome subsite (<http://maize-pangenome-ensembl.gramene.org>), will feature uniformly annotated and comprehensively mapped NAM founders genomes for studying structural variation impacting CNV and PAV. Gramene is supported by an NSF grant (IOS-1127112), and partially from USDA-ARS (1907-21000-030-00D).

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## Mining transcriptional cis-regulatory modules in the maize genome

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Transcription factors (TFs) recognize short DNA sequence motifs in regulatory regions of their target genes and thus control the gene expression changes responsible for plant developmental programs and environmental responses. To expand our currently limited view of the functional non-coding space in maize, we are using DAP-seq, a cost-effective in vitro alternative to ChIP-seq, to map TF binding events. We have generated high-quality B73 DAP-seq datasets for 45 maize TFs from 12 different families. We observe that many TFs often bind within close proximity to one another forming putative cis-regulatory modules (CRMs; also commonly referred to as enhancers). These CRMs frequently overlap with regions of accessible chromatin determined using ATAC-seq and can be located both proximally and distally at regions >40kb from gene bodies. Such proximal and distal CRMs were observed in several plant architecture-related genes including *TEOSINTE GLUME ARCHITECTURE1*, *GRASSY TILLERS1*, *TEOSINTE BRANCHED1*, and *UNBRANCHED2*. This approach is providing a highly integrated view of how multiple TFs contribute to the control of certain transcriptional programs.

A significant percentage of trait-associated variants lie within non-coding regions and likely affect TF binding. By performing DAP-seq with genomic DNA from different maize inbred backgrounds (B73 and Mo17), we have identified both conserved and background-specific cis-regulatory modules, allowing us to explore how cis-regulatory variation contributes to phenotypic diversity in maize. Pairing such information with QTL data further provides testable hypotheses regarding the molecular cause of known traits. For example, we observe several shared and Mo17-specific TF binding events within a previously described QTL for increased *BXI* expression, a gene involved in the biosynthesis of the herbivore resistance compound DIMBOA. Precise CRISPR-based editing of these TF binding events and those described above is underway to dissect the molecular mechanisms that drive plant transcription and better understand how regulatory sequence changes impact distinct plant phenotypes.

Funding acknowledgement: National Science Foundation (NSF)

P56 

## Predicting differential expression of drought-associated genes across Andropogoneae

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With the average global temperature predicted to rise over the next century, breeders will need to develop crops that can not only thrive in these conditions, but feed a growing population with less resources. Maize, sorghum, and their relatives in the Andropogoneae tribe are among the most productive and widely-adapted plants in the world, collectively representing over a billion years of evolution. Drought is already a critical threat to rainfed maize production systems across the world and climate change will potentially worsen its impact. Members of Andropogoneae have independently adapted to both high temperatures and low precipitation and utilize C4 photosynthesis, a significant contributor to their productivity. We will present on using machine learning to develop models that can predict the direction of expression change in response to drought conditions directly from sequence. Previously, we examined differential expression in the NAM founders under no-water conditions (plants grown in growth chambers for three weeks and then water was withheld for five days), RNAseq was performed on leaf tissue collected on day one and three of drought treatment. Using this data, we have trained machine learning models to predict differential expression directly from sequence. Given a genotype, the models try to predict whether a gene will be upregulated, downregulated, or have no expression change. These maize-trained models will be applied to incoming and existing genome assemblies of Andropogoneae to identify drought response loci and explore differences in the genetic architecture of drought tolerance among the tribe members.

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

P57 

## Predicting functions for novel maize orphan genes using network and clustering analysis

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Gene functions are generally predicted either by comparing them against the genes of known function or by finding functional motifs in the sequence. Although many of the genes, due to having a common ancestry, can be annotated this way, many other genes in maize remain unannotated. The primary reason for not having functional assignments are that the genes are orphans (genes with no known homology to closely related species) or that they lack any motif inferring function. Here, we analyze maize transcripts, including known genes and candidate genes, extracting expression data from over 3000 NCBI-SRA runs, and building a co-expression network from the normalized expression data. We cluster the transcripts based on their expression values using different methods. For these clusters we measure the enrichment for GO terms and KEGG assignments to estimate the biological significance of the clusters, and perform statistical analysis, and metadata mining. We visualize the transcripts using MetaOmGraph ([http://metnetweb.gdcb.iastate.edu/MetNet\\_MetaOmGraph.htm](http://metnetweb.gdcb.iastate.edu/MetNet_MetaOmGraph.htm)). The preliminary results provide a rich context for experimental biologists to make novel, testable hypotheses as to the function of as-yet-unannotated transcripts and design experiments to elucidate their role.

Funding acknowledgement: National Science Foundation (NSF)

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## **Preliminary results from the GBS analysis of 2,236 inbred maize lines in Eastern Europe**

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Romania was ranking 4th in terms of maize production in the 80s, surpassing USSR and the only other European country present in the world top 10, France (source: FAOSTAT 1978-1980). This was mainly the result of intensive breeding programs that have started in the 60s, which involved systematic collection of seeds from open pollinated populations followed by extraction of inbred lines. Since then, a lot of foreign germplasm has been brought-in especially from the Americas. This led to a present-day mix in the germplasm that we have probed previously using SSR molecular markers on 90 inbred lines (*Suteu et al., 2013*). We reported a high allelic richness and a population of inbred lines that didn't match any of the gene pools defined by inbred lines like Fv2, Lo3, D105, C103, B73, Oh43, and W153R. Recently, we have just finished the analysis of another 250 inbred lines, confirming the previous findings.

Here, we present preliminary results from a GBS approach in understanding the genetic makeup of 2,236 inbred lines, including 600 from the Republic of Moldova, where germplasm from the former Soviet republics has been intensively used in breeding programs, and 100 from Serbia, as an exponent of former Yugoslavia. When completed, the study will provide a comprehensive genetic structure of the inbred lines, grouped into heterotic groups, which will be later used in breeding programs nationwide and beyond for creating superior hybrids.

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P59

## **Prophyler: a do-it-yourself image-based phenotyping and analysis platform based on community cyber infrastructure**

(submitted by Nathan Miller <[ndmiller@wisc.edu](mailto:ndmiller@wisc.edu)>)

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Image-based plant phenotyping requires data acquisition and analysis, which are inherently coupled. Continued community efforts have focused on development of software, hardware, and languages to increase the throughput of imaging methods. Often, this leads to high-performance phenotyping focused on as much automation as possible, which results in specialized and centralized data gathering hardware. The price and expertise associated with this limits widespread community adoption and supports a hub-and-spoke service model. Alternatively, distributed high-throughput phenotyping focuses on autonomy and demands a solution easily implemented that results in a decentralized network of users. To this end we have designed a platform focusing on stability, flexibility, and accessibility in metadata gathering, trait extraction, and analysis. In our system, plants are grown in a controlled environment setting and manually placed against a backdrop that includes machine-readable user defined metadata sheet that is embedded in the image. Side-view RGB images are automatically acquired with consumer-grade cameras and can be pushed to CyVerse-managed data-storage infrastructure. Machine learning algorithms robustly segment each image into background, plant, and metadata components. The latter two components are analyzed with custom software that executes without user interaction and in parallel. The method was developed to measure maize growth and architecture and quantifies traits from images such as height, width, stem diameter, and center of mass. Prototypes of the Prophyler tool have been used to quantify growth and architecture phenotypes of mapping populations of maize subjected to abiotic stress across multiple institutions. In addition, we have also developed an R Shiny application for initial data quality control and analysis. The metadata sheet creation, trait extraction, and analysis functions are deployed on NSF-funded high-throughput computing resources accessed via a CyVerse Web interface. The simplicity of the image-acquisition hardware and the web-based trait and analysis functions make this image-based phenotyping method broadly accessible.

Funding acknowledgement: National Science Foundation (NSF)

P60

## **Repetitive DNA content in the maize genome is uncoupled from population stratification at SNP loci**

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Repetitive DNA is a major component of plant genomes and is thought to be a driver of evolutionary novelty. Describing variation in repeat content among individuals and between populations is key to elucidating the evolutionary significance of repetitive DNA. However, the cost of producing reference genomes has limited large-scale intraspecific comparisons to a handful of model organisms where multiple reference genomes are available. We examine repeat content variation in the genomes of 95 elite inbred maize lines using graph-based repeat clustering, a reference-free and rapid assay of repeat content. We examine population structure using genome-wide repeat profiles and demonstrate the stiff-stalk and non-stiff-stalk heterotic populations are homogenous with regard to global repeat content. In contrast and similar to previously reported results, the same individuals show clear differentiation, and aggregate into two populations, when examining population structure using genome-wide SNPs. Additionally, we develop a novel kmer based technique to examine the chromosomal distribution of repeat clusters *in silico* and show a cluster dependent statistically significant association with gene density. Our results indicate that repeat content variation in the heterotic populations of maize has not diverged and is uncoupled from population stratification at SNP loci. We also show that repeat families exhibit divergent patterns with regard to chromosomal distribution, some repeat clusters accumulate in regions of high gene density, whereas others aggregate in regions of low gene density.

P61

## Root growth differences found between historical and modern density adapted maize

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We face the daunting need to double food production sustainably within the next 30 years. Crop production is often maximized by selection for increased plant density per acre, but this effort is limited by detrimental effects of competition, which vary across major crop species. In the past century, U.S. maize grain yield increased six-fold, largely attributed to selection of improved performance of hybrids in progressively dense sowing practices. Changes in leaf angle and anthesis-silking interval are examples of specific traits associated with germplasm adapted for densely planted fields, however there have been few empirically derived underground factors found, such as root architecture, root-root interactions, or microbiome associations. This study analyzes germplasm from historically important populations: Iowa Stiff Stalk Synthetic (BSSS), Corn Borer Synthetic (BSCB), and their hybrid (HY). The BSSS and BSCB populations have been maintained at non-selected cycle 0 (C0), and along many generations of selection for yield in progressively denser environments, including cycle 17 (C17). Our research combines modern, high-resolution X-Ray Computed Tomography (XCT) with traditional root excavation and 2-D imaging, to assay three time points at week 4, 6 and 9 (flowering) across six genotypes, at two planting densities (3 plants/m<sup>2</sup> and 9 plants/m<sup>2</sup>). Above-ground tissue and excavated roots were dried and weighed in addition to obtaining leaf and root whorls counts for each time point. We found in general that modern corn roots tend to be smaller in size for area and by dry weight, the exception being C17 Hybrids in high-density. We also found root shape largely depended on neighboring Cycle in high density.

Funding acknowledgement: National Science Foundation (NSF)

P62 

## Sequence, assembly and annotation of Bayer Crop Science's maize inbred line LH244; a new Resource for maize genetics and transformation

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Access to elite, transformable germplasm is required to design and maintain transformation pipelines. Product pipelines typically use transformable germplasm to initially introduce the novel variant, which is then crossed into broad, diverse germplasm lines relevant to the geographies where the product will be grown. Effective transformation pipelines are valuable for product development in the Ag industry but are also important for serving the scientific community by enabling basic science research through gene and pathway discovery and characterization. Bayer Crop Science, in collaboration with NRGene and the University of Wisconsin, reports the release of the LH244 inbred maize transformation line germplasm and assembled reference genome to academic research communities. The germplasm will be released to public seed stock centers and the assembled, annotated genome and a physiological description of the line will be published, and resources for efficient transformation will be available to the University of Wisconsin Crop Innovation Center. LH244 is a commercially relevant inbred line that is readily transformable, thus making it a complete resource for genomic and genetic exploration. In this talk, we will share insights into the unique features of the LH244 genome, transformability and physiology that make it a foundation resource for the maize genetics community.

Funding acknowledgement: Bayer Crop Science



P63

## Spatio-temporal transcriptional dynamics of maize long non-coding RNAs responsive to drought stress

(submitted by Xia Zhang <[zhangxia@caas.cn](mailto:zhangxia@caas.cn)>)

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Drought is a major abiotic stress that adversely impacts plant growth and productivity. Recently, long non-coding RNAs (lncRNAs) have emerged as important regulators in plant development and stress response. To define the dynamic spatiotemporal expression of maize lncRNAs and their functional landscape in drought response, we performed a genome-wide lncRNA transcriptional analysis using an expanded series of maize samples collected from three distinct tissues (ear, leaf, tassel) spanning four developmental stages (V12, V14, V18, R1). By applying a stringent filtering pipeline, we identified a full set of high-confidence lncRNAs and 1,535 lncRNAs were characterized as drought responsive. Investigations on the genomic structure and expression pattern show that lncRNA structures were less complex than protein-coding genes, showing shorter transcripts and fewer exons. Comparing with protein-coding genes, drought-responsive lncRNAs exhibited higher tissue- and developmental-specificity. Temporal expression patterns of drought-responsive lncRNAs at different developmental stages identified the reproductive stage R1 was the most sensitive growth stage with more lncRNAs showing altered expression upon drought stress. Furthermore, lncRNA target prediction revealed 653 potential lncRNA-mRNA pairs, among which 124 pairs function in cis-acting mode and 529 in trans. These targets were functionally enriched in stress response category related to oxidoreductase activity, water binding, and electron carrier activity. We also uncovered multiple promising targets of drought-responsive lncRNAs, including the V-ATPase encoding gene, *vpp4*. These findings extend our knowledge of lncRNAs as important regulators in maize drought response.

Funding acknowledgement: National Key Research and Development Program of China (2016YFD0101002)

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## Structural variation analysis in the Wisconsin Diversity Panel: pros and cons of using multiple de novo genome assemblies

(submitted by Patrick Monnahan <[pmonnahan@gmail.com](mailto:pmonnahan@gmail.com)>)

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The increasing number of reference genome assemblies available to maize researchers represents both an opportunity and a challenge with regard to the detection of structural variation. Ideally, the consistency of a particular variant across reference genomes, in terms of location, genotype, etc., ought to serve as corroborating evidence for structural variant identification. However, imperfect assemblies and/or annotations along with the challenge of correctly linking genes/coordinates across references may obscure our ability to appropriately validate certain variants. Here, we compare/contrast structural variation detected in ~100 inbred lines from the Wisconsin Diversity Panel when mapped to each of 5 reference assemblies: B73, PH207, PHB47, W22, and Mo17. Short-read sequencing at 10-40x depth was used to detect variants using two programs: LUMPY and Genome STRiP. Idiosyncrasies of particular assemblies were observed throughout the variant filtration process. For example, a greater number of variants tend to be called with respect to B73 and a higher proportion of these are retained following filtration. We consider the implications of these reference idiosyncrasies with respect to linking genotype to phenotype in an association framework.

Funding acknowledgement: National Science Foundation (NSF)



P65 

## The development of computational resources for association studies in maize and sorghum

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Association studies using biological intermediates (i.e. endophenotypes) can produce large amounts of data. Therefore, questions arise with how to efficiently analyze, store, query, and link this data to other sources of information and analytical software for biological insight. Due to the overall size and complexity of this data schema, traditional relational databases can become inefficient in terms of speed and performance. Here, we introduce a Java implemented graph database approach for linking variant loci with endophenotypic traits and regulatory information in maize and sorghum. This solution provides faster querying and more intuitive methodologies for determining relationships between sources of biological data. We also introduce rTASSEL: an integrative R framework for the popular TASSEL (Trait Analysis by aSSociation, Evolution, and Linkage) software. This integration combines the exceptional analytical performance of TASSEL with R based data objects for downstream analysis and visualization using the R framework.

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

P66 

## The transcriptional landscape of diverse maize genotypes

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A gene's transcriptional expression pattern provides insight into how polymorphism influences an organism's response to environmental input. Genetic differences can lead to variation in gene expression across lines and environments as well as within specific tissues. Here, we use a computational framework called **Camoco** to compare and contrast the transcriptional landscape of 4 different maize primary tissues (leaf, root, shoot, and seed) representing between 2 and 4 datasets per tissue. Within each of these tissues specific datasets are gene expression data from between 21 and 201 different diverse maize accessions. Gene expression profiles are compared within each tissue to build a total of 13 tissue-specific, genotypically diverse gene co-expression networks.

In these networks, nodes represent genes while edges represent similarity of gene expression, in each tissue, across different maize accessions. The different tissue networks show significant co-expression for between 34% and 75% of GO terms. Multi-network GO enrichment identifies a subset of GO terms containing genes that are either ubiquitously co-expressed in all tissues indicating steady-state or "house-keeping" processes (e.g. "nucleosome assembly" and "ribosomal subunit"). Similarly, tissue specific GO terms were identified showing strong co-expression in only a single tissue source. As expected, tissue specific terms included categories such as "response to water" and "ion binding" for the root networks, "transmembrane transporter activity" in the shoots, and "photosystem" and "pigment metabolic process" in the leaves. To complement this directed, ontology based approach, an un-directed approach not relying on annotations was employed. Strongly co-expressed gene clusters defined by Markov Clustering (MCL) identifies sets of unannotated genes showing tissue specific patterns of co-expression. Coupling together these wide-reaching transcriptional datasets with our network analysis tool, **Camoco**, we mapped the transcriptional landscape of maize accessions showcasing links between genetic diversity and gene expression.

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

P67

## The whole genome assembly and population genetics of sweet corn

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Sweet corn is a specialty crop and one of the most popular vegetables in the United States. Despite advances in genome assembly and annotation in multiple lines of field corn, a reference genome of for sweet corn is currently unavailable. In this project, we sequenced a sweet corn inbred line, Ia453 with the mutated shrunken 2 allele (Ia453-sh2). This mutation increases sugar content and is present in the vast majority of commercial hybrids developed for the fresh market. The genome was sequenced using single-molecule real-time sequencing technologies. We generated 59.79-fold whole-genome shotgun sequence coverage on a PacBio Sequel instrument. The genome was assembled into 15,701 contigs with initial N50 of 0.32 Mb. Currently, the draft assembly is being polished with short read data. In parallel, Chicago library scaffolded with the HiRise software pipeline will be used to increase the contiguity of scaffolds. Genes of the Ia453 genome were annotated using a Maker-P pipeline including single-molecule Iso-seq transcript sequencing data. In total, 43,186 genes were identified as 'working gene set'. In addition to the reference genome, we performed population genetics analysis on 590 sweetcorn lines and 273 field corn lines genotyped with genotyping by sequencing. Results show regions in the sweet corn population under strong evidence of selection. Chromosome 4, which contains the sugary 1 gene has a 50 Mb window around sugary1 (*su1*) and 30 Mb window across the centromere with very low. Our study provides a high-quality reference genome of sweet corn for further follow-on studies as well as a set of target regions potentially relevant for the traits that are specific to sweet corn and are under selection when compared with field corn.

Gene / Gene Models described: *su1*; GRMZM2G138060

Funding acknowledgement: United States Department of Agriculture (USDA)

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## Time-series analysis of maize transcriptomes under drought stress

(submitted by Cheng He <[ksuhecheng90@gmail.com](mailto:ksuhecheng90@gmail.com)>)

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Maize production is threatened by drought stress worldwide. Plant physiological responses to drought stress are driven by regulation of gene expression. While drought-responsive genes (DRGs) have been identified in maize, regulation patterns of gene expression along with progressive water deficits remain to be elucidated. In this study, we generated time-series transcriptomic data of the maize inbred line B73 under both well-watered and drought conditions. Comparison between the two conditions identified 8,626 DRGs, of which 2,840 and 3,699 were up- and down-regulated along with drought stress, respectively. Early, middle and late drought stage DRGs were also identified. Gene ontology enrichment analysis indicated that different groups of genes were highly regulated at three stages. Upon re-watering drought-stressed seedlings, expression of most DRGs at early and middle drought stages, particularly early DRGs, was restored to levels near that of well-watered seedlings. Genes with copy number variation among diverse maize lines were enriched in early and middle DRGs, as compared to late DRGs, and exhibited a stronger association with drought tolerance based on genome-wide association study (GWAS) results. In addition, examination of expression correlation between small RNAs (sRNAs) and genes (mRNAs) identified 412 negatively correlated sRNA-gene pairs, representing potential genes targeted by corresponding sRNAs. Among them, 15 pairs were identified from drought responsive sRNAs and DRGs, including two glutamine family genes, *gln2* and *gln6*, and a CBF1 ortholog. In summary, characterization of dynamic gene responses to progressive imposition of drought stresses indicates important adaptive roles of early and middle DRGs, as well as the role of sRNAs in gene expression regulation upon drought stress.

Gene / Gene Models described: *gln2*, *gln6*; GRMZM2G024104, GRMZM2G050514

Funding acknowledgement: National Science Foundation (NSF)

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## Time-series GWAS using non-parametric regression and machine learning

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Genome sequencing and genotyping technologies have facilitated the success of genome-wide association studies (GWAS) in the past decade. Compared to genomics and genotyping, until recently approaches to phenotyping had remained reasonably stable for quite some time. However, the role of collecting trait data as the primary bottleneck in quantitative genetics has spurred an interest in new high throughput approaches to plant phenotyping, including image-based phenotyping. In principle, image-based phenotyping should be non-destructive, high throughput and less labor intensive than conventional phenotyping. However, several challenges remain including the extraction of numerical traits from thousands or tens of thousands of images and developing appropriate statistical approaches to linking genotype to traits which change dynamically over time. Here we use hyperspectral image data generated from the sorghum association panel (SAP), a set of ~400 diverse sorghum lines, during the transition from vegetative to reproductive development to perform a time-series GWAS analysis. After distinct plant organs were identified in each hyperspectral image, measurements were extracted for each observed timepoint and nonparametric regression was employed to estimate missing timepoints for each individual. The timing of vegetative to reproductive transition was determined using a convolutional neural network (CNN) trained using images of sorghum plants with and without panicles. Our results suggest that time-series GWAS leveraging different statistical and machine learning approaches can significantly increase the power to identify causal variants in associated traits relative to single timepoint GWAS at a mature developmental stage.

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## Tools for single-cell RNA-seq in maize

(submitted by Brad Nelms <[bnelms.research@gmail.com](mailto:bnelms.research@gmail.com)>)

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Single-cell RNA-sequencing is a powerful tool to identify cell sub-populations and reconstruct developmental pathways. We present two techniques to facilitate single-cell RNA-seq for maize research. One challenge of adapting single-cell RNA-seq to plant biology is the difficulty of digesting the cell wall to release cells, and the fragility of the resulting protoplasts. In the first technique, we show that pre-fixing plant tissues prior to cell wall digestion makes it possible to use harsher conditions that result in consistent and quantitative release of cells; it also halts biological changes during sample handling and maintains cell morphology to aid in the identification of plant cell types. In the second, we present a statistical method to quantify the rate of gene expression change over time from single-cell data, called ‘pseudotime velocity’. Cell differentiation is often modeled as the passage through a series of discrete cell states, but it is an open question of how discrete these cell-state transitions truly are: does differentiation always occur through rapid transitions separating meta-stable states? or do cells sometimes differentiate through a continuum of intermediates? Pseudotime velocity is the first approach to allow for an estimate of statistical uncertainty (by bootstrapping) when inferring the rate of expression change from single-cell data. We applied this method to many published single-cell RNA-seq datasets and found that both discrete and continuous transitions are common during cell differentiation. By correlating these features with known developmental events, we propose biological explanations for why differentiation might occur rapidly at some times and more slowly at others.

Funding acknowledgement: National Science Foundation (NSF)

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## **Transcriptomic response to divergent selection for flowering time in maize reveals convergence and key players of the underlying gene regulatory network** (submitted by Maud Tenaillon <[maud.tenaillon@inra.fr](mailto:maud.tenaillon@inra.fr)>)

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Artificial selection experiments are designed to investigate phenotypic evolution of complex traits and its genetic bases. Here we focused on flowering time, a trait of key importance for plant adaptation and life-cycle shifts. We undertook divergent selection experiments (Saclay DSEs) from two maize inbred lines. After 13 generations of selection, we obtained a time-lag of roughly two weeks between Early- and Late- populations. We used this material to characterize the genome-wide transcriptomic response to selection in the shoot apical meristem (SAM) before, during and after floral transition in realistic field conditions during two consecutive years. We validated the reliability of performing RNA-sequencing in uncontrolled conditions. We found that roughly half of maize genes were expressed in the SAM, 59.3% of which were differentially expressed. We detected a majority of genes with differential expression between inbreds and across meristem status, and retrieved a subset of 2,451 genes involved in the response to selection. Among these, we found a significant enrichment for genes with known function in maize flowering time. Furthermore, they were more often shared between inbreds than expected by chance, suggesting convergence of gene expression. We discuss new insights into the expression pattern of key players of the underlying gene regulatory network including ZCN8, RAP2.7, ZMM4, KN1, GA2ox1, as well as alternative scenarios for genetic convergence.

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## **Understanding evolution of the complex NLR immune gene family in panicoideae**

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Devastating diseases that affect crops can be prevented through the activation of the plant immune system. Nucleotide Binding Leucine Rich Repeat (NLR) proteins are essential for the recognition of pathogen derived molecules within a plant cell. These proteins recognise and trigger an immune response upon identifying presence of a pathogen. The number of NLRs varies from approximately 190 to over 2,000 in the Poaceae family alone. Using publicly available genomes and phylogenetic methods, we identified an unexpected reduction in NLR number across many NLR clades in Maize.

To further our understanding of processes causing a reduction in NLR number we choose the model species Maize. We were interested to identify if the low number of NLRs seen in maize comparative to other Panicoideae and Poaceae is due to domestication, speciation or inbreeding. To answer this question we designed a sequence capture for NLRs and chromosomal markers. We subsequently collected diverse germplasm from wild, landrace and elite lines of Maize, Teosinte, Sorghum and Setaria. Genomic DNA was extracted and probes we designed captured NLR and marker genes of interest before PacBio sequencing.

Current work aims to assemble and annotate the NLRs from the sequencing. Upon doing this we will identify selective pressures acting on the NLRs identified and the presence absence variation within and among species. This dataset will be a resource for the community in trying to increase diversity in resistance to rapidly evolving pathogens.

Funding acknowledgement: European Research Council (ERC), Biotechnology and Biological Sciences Research Council(BBSRC)

P73

## Understanding the relationship between gene co-expression networks and trait variation

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Heterosis, the superior performance of hybrids relative to its parents, is considered the major driver of yield increase in maize in the last decades, but its molecular basis is still not very well understood despite extensive research. Many mechanisms are thought to contribute with heterosis, including differential gene expression between inbred lines and hybrids. Thus, integrating gene expression from RNA-seq data with phenotypic data of both inbreds and hybrids may be useful for identifying genes related to heterosis. One approach to tackle this issue is by creating gene co-expression networks and correlating networks of genes with trait variation. In this exploratory study, we are using Camoco to generate gene co-expression networks from RNA-seq data of five different tissues (leaf, internode, seedling, endosperm, and root) harvested from a diverse panel of maize inbreds and their hybrids offspring. Our goal is to answer two questions. Are there gene modules that correlate with trait values in inbreds and/or hybrids? If so, is there consistency across inbreds and hybrids. Are there gene modules in hybrid networks that correlate with the percent heterosis for a trait? Results of this study will contribute to our understanding of the relationship of molecular and phenotypic variation for heterosis.

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

P74 

## Updating the maize TFome and the GRASSIUS database: Resources for regulomics in the grasses

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Gene regulatory networks (GRNs) are central to all cellular processes. Deciphering GRNs at the molecular level is key to understanding and manipulating important agronomic traits for improved food and fiber production. To advance the study of regulomics in cereals we previously developed and released the Maize Transcription Factor ORFeome (TFome). The advent of the most recent Maize genome (AGP B73v4) has permitted increasing the number of predicted TF genes to about 3400. The availability of many RNA-Seq datasets permits these gene models to be corrected and assigned to appropriate TF families. Combined with synteny data, these gene models are being evaluated for likely duplicate, overlapping, or loss of function. We are now expanding the TFome collection beyond the original 2,017 unique Transcription factor (TF) and CoRegulator (CR) gene that were cloned in recombination-ready vectors (Burdo et al., *The Plant Journal*. 2014 80:356-66). These new clones are being made available through the Arabidopsis Biological Resource Center (ABRC). In parallel, we are updating the GRASSIUS ([grassius.org](http://grassius.org)) knowledgebase which was developed to serve as hub for gene regulatory information for the grasses. GRASSIUS consists of three interlinked databases that contains a collection of TFs classified into different families (GrassTFDB); transcriptional co-regulators (GrassCoRegDB); and promoter sequences (GrassPROMDB) for maize and other grasses. GRASSIUS is home to the maize TFome and is being updated to host experimentally determined TF/coregulator protein-DNA interactions (Yang et al., 2017. *Mol Plant*, 10:498–515) and ongoing annotation of maize transcription start sites (TSSs) derived from Cap Analysis of Gene Expression (CAGE) experiments (Mejia-Guerra, et al., *Plant Cell*. (12):3309-20). The utility of GRASSIUS combined with the maize TFome to the scientific community is to accelerate elucidation of regulatory mechanisms that are vital for engineering cereal crops with improved agronomic traits. This project is funded by NSF grant IOS-1733633.

Funding acknowledgement: National Science Foundation (NSF)



P75 

## **Zea Lip: A developmental atlas of glycerolipid species in diverse maize material**

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Glycerolipids are the predominant building blocks of plant membranes and are essential for plant growth and development. They are involved in a variety of signaling and their relative abundances can change in response to several environmental factors. In an effort to catalog the composition of glycerolipids in an important crop like maize, we used ultra-high performance liquid chromatography coupled with quadrupole time of flight mass spectrometry to characterize the glycerolipid profile of samples of maize plants collected at 10 different vegetative developmental stages and 6 different leaf ages. We analyzed three different genotypes: B73, a temperate inbred, CML312, a tropical inbred and Palomero Toluqueño, a landrace from the Mexican highlands. In total 150 samples were analyzed and we could identify around 120 glycerolipid species. Overall, genotype was the major driver of glycerolipid differences. Phosphatidylcholine and lysophosphatidylcholine genotypic differences were particularly high. We designed a web interface to easily browse and compare glycerolipid levels across tissues and genotypes that can be accessed at [github.com/rr-lab/zea\\_lip/](https://github.com/rr-lab/zea_lip). We plan to keep adding data from other genotypes to this database and make this resource available to the maize community.

Funding acknowledgement: Conacyt, Cinvestav

P76

## **Achieving genome editing in recalcitrant maize lines by biolistic delivery of CRISPR reagents and morphogenic genes**

(submitted by Morgan McCaw <[mccawm@iastate.edu](mailto:mccawm@iastate.edu)>)

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Fast-Flowering Mini-Maize (FFMM) is an excellent model plant for maize research because of its short stature, more efficient use of greenhouse space, and rapid generation time. However, FFMM is not amenable for genetic transformation using a standard maize transformation protocol. Here we report an effort utilizing a second-generation *Baby boom* and *Wuschel2* morphogenic genes construct (Dev2) from Corteva for achieving CRISPR/Cas9-mediated, targeted mutagenesis of *Glossy2* (*Gl2*) in FFMM-A. Co-bombardment of immature embryos with a Dev2 plasmid (PHP79066) and a second plasmid carrying Cas9 with a guide RNA targeting *Gl2* produced clonal plants with a homozygous 1 bp insertion (A) mutation at the target site. Consistent with the genotyping results, the *gl2* knock-out mutant produced a phenotype that can be easily visualized by the adherence of water droplets to the leaf surface.

Gene / Gene Models described: *gl2*; GRMZM2G098239, Zm00001d002353

P77 

## **Towards establishment of a transformable model maize line: Breeding type II embryogenic callus response into fast-flowering mini-maize**

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Fast-Flowering Mini-Maize (FFMM) is an excellent model plant for maize research because of its short stature, more efficient use of greenhouse space, and rapid generation time. When introduced to the maize community, FFMM was not capable of routine genetic transformation; this limited its usefulness in projects requiring a transgenic component, such as CRISPR/Cas9 gene editing. In an effort to establish a transformable FFMM, we back-crossed one line, FFMM-A, with the readily transformable maize genotype Hi II Parent A. Following several generations of self-pollination, we have selected lines strongly resembling FFMM-A that are capable of forming embryogenic callus that can be regenerated into fertile plants. These lines were capable of producing Type-II callus from immature embryos on N6 media at high frequency in self-generation II through IV, though a reduction in Type-II callus production frequency and vigor of said callus has arisen during inbreeding. These lines, up to self-generation VII, still respond vigorously in tissue culture with a different formulation of media. The development of a robust protocol for transformation of FFMM with this media is ongoing.

P78

## **A Hardier Crop: Identification of abiotic stress responsive genes in Zea mays (maize) dependent on MOP1-mediated epigenetic regulation and the plant hormone ABA.**

(submitted by Rachel Calder <[calder4@uw.edu](mailto:calder4@uw.edu)>)

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Zea mays (maize, corn) is an essential crop plant; important to global agriculture and the U.S. economy. However, maize productivity and yield can be drastically affected by abiotic environmental stress. Therefore, a priority for many plant breeding programs is to select for crops displaying phenotypic traits of enhanced tolerance to abiotic stress.

A subset of abiotic stresses induce the plant hormone, abscisic acid (ABA). This hormone acts as an inhibitor in certain pathways and can prevent germination and development of the plant through alterations in the expression of stress responsive genes.

In maize, the mediator of paramutation1 (MOP1) gene encodes an RNA-dependent RNA polymerase that functions in the RNA-directed DNA methylation (RdDM) pathway. Regulated DNA methylation is essential for normal plant development. A mutation in the MOP1 gene (mop1-1; Dorweiler et al., 2000) results in the loss of DNA methylation which in turn causes a variety of genes to be expressed abnormally.

I will determine how a mutation in a mop1-1 affects RNA expression under abiotic stress by conducting a computational analysis of multiple RNA-seq datasets of stress-treated maize. I will compare the RNA-seq data from the Madzima (UWB) and McGinnis (FSU) labs (Vendramin et al. in prep) of WT Mop1 and MUT mop1-1 plants treated with ABA or control (no ABA treatment) with the dataset from Li, P. et al.'s published data (2017), of WT maize plants treated with heat, cold, drought, salinity, and control (no stress treatment). Genes commonly down-regulated in the four stresses and in MOP1 WT ABA, but up-regulated in mop1-1 ABA will represent genes potentially silenced under stress that require MOP1 for DNA methylation. The presence of these genes in the given stress treatment allows us to identify the abiotic stresses that require ABA and MOP1 epigenetic regulation.

Funding acknowledgement: Mary Gates Foundation



P79

## **A novel dwarf mutant with a gain-of-function mutation in a glutamate receptor gene**

(submitted by Amanpreet Kaur <[kaur60@purdue.edu](mailto:kaur60@purdue.edu)>)

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Glutamate receptors (GluRs) are ligand-gated ion channels well known for their role in neurotransmission. They allow the flow of cations such as Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> across membranes in response to glutamate binding. Homologs of GluRs are widely present in plants, with maize and *Arabidopsis* containing 18 and 20 members, respectively. They are involved in a range of activities in plants, including signaling in light responses, plant defense, long-distance wound responses, drought tolerance, and C:N ratio sensing. None of the studies so far have indicated any role for these receptors in plant architecture modulation. We identified a dominant dwarf (named D13) in an M1 population of B73 generated by EMS mutagenesis. Map based cloning has identified a G to A change in a GluR gene as the top candidate for the D13 phenotype. This G to A polymorphism results in the substitution of an amino acid that is totally conserved in all GluRs. The RNA-seq data indicates that D13 is unique and unlike any of the known dwarf mutants defective in the biosynthesis or signaling of key phytohormones including gibberellins and brassinosteroids. The mutant phenotype however is unstable, as it is significantly impacted by the environment (especially light duration). The genetic background also influences D13 considerably. Efforts are underway to validate and clone the gene, as well as to characterize D13 with respect to its physiological, molecular and metabolomic phenotypes.

P80 

## **A topographical approach to elucidate sugar metabolism in the developing maize kernel**

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Sugar uptake, metabolism and conversion into biomass components are the base for kernel growth. Current kernel models suggest that sucrose (major photosynthate) is supplied to kernels, where it moves from pedicel toward BETL cells. Before entering the endosperm Suc is partially hydrolyzed to hexoses, and upon import sucrose is re-synthesized. We are examining exactly where (and why) this happens. For this, we investigate developing kernels using magnetic resonance imaging (MRI) and visualize their internal structures non-invasively. We further apply mass spectrometry-based metabolite profiling and use a novel sugar imaging tool (Guendel et al., *Plant Physiology* 2018/178). The infrared-based, microspectroscopic method allows for the quantitative visualization of sucrose at a microscopic level of resolution (~12 µm). The method has been used to visualize sucrose distribution across the kernel at distinct developing stages for genotype B73. We discuss the relevance of the method for studies on kernel development, carbon allocation and storage metabolism in the context of crop improvement.

Funding acknowledgement: Deutsche Forschungsgemeinschaft (DFG)

P81

## **A vacuolar K<sup>+</sup>/H<sup>+</sup> antiporter gene ZmNHX3 can improve the saline and alkali tolerance of maize**

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The tonoplast and plasma membrane localized sodium (potassium)/proton antiporters have been shown to play an important role in plant resistance to saline and alkali stress. In this study, we cloned ZmNHX3 of putative tonoplast-associated Na<sup>+</sup>/H<sup>+</sup> antiporters of higher plants, and investigated its functions in Arabidopsis. Experiments with ZmNHX3-GFP fusion protein in tobacco protoplasts indicated that ZmNHX3 is mainly localized to vacuolar membrane, with a minor localization to pre-vacuolar compartments (PVCs) and endoplasmic reticulum (ER). ZmNHX3 were studied using 35S-driven ZmNHX3 over-expression in Arabidopsis plants. RT-PCR analyses revealed that ZmNHX3 is highly expressed in seedlings under saline and alkali treatment. The genotypes indicated that it can increase the tolerance to saline and alkali during the seedlings, molecular assistant selection(MAS) may represent a feasible way to improve the resistance to saline and alkali of maize.

Gene / Gene Models described: *ZmNHX3*; GRMZM2G063492

Funding acknowledgement: Jilin Provincial Science and Technology Key Project(20170204007NY)

P82 

## **Allelic interactions of induced and natural variation at oil yellow1 impact flowering time in maize**

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The first committed step of chlorophyll biosynthesis involves the conversion of protoporphyrin IX (PPIX) to magnesium-PPIX by magnesium chelatase (MgChl), a hetero-oligomeric enzyme complex consisting of three subunits (I, D, and H). In maize, *oil yellow1* (*oy1*) encodes subunit I of MgChl. Using a semi-dominant mutant allele *Oy1-N1989* as a reporter of leaf greenness, we previously identified a single Mendelian modifier, *very oil yellow1* (*vey1*), in the maize association panel that encodes a putative cis-acting expression polymorphism at *oy1*. Here we demonstrate that reduced chlorophyll accumulation due to the *Oy1-N1989* mutation delays flowering time, and its suppression by *vey1* accelerates flowering of the mutants. These changes were concomitant with changes in carbon metabolism as well as other developmental and physiological processes, such as tassel architecture and leaf senescence, that are affected by sugar availability. Suppression of total photosynthate accumulation by mechanical removal of leaves also delayed flowering time, consistent with an integrative measure of carbon assimilation determining the linkage between energy status and flowering time in maize. In addition to the effect of *vey1*, known loci modulating flowering time also displayed *Oy1-N1989*-dependent genetic effects on flowering. The most notable among these differences were alleles at *zea centroradialis8* (*zcn8*), which encodes for the maize florigen. Alleles of *zcn8* showed epistatic interactions with the *Oy1-N1989* mutant allele. These findings provide an impetus to explore the nature of the linkage between carbon capture and known core regulators of flowering in maize.

Gene / Gene Models described: *oy1*, *zcn8*; GRMZM2G419806, GRMZM2G179264

Funding acknowledgement: National Science Foundation (NSF), USAID

P83

## **An *Agrobacterium*-delivered CRISPR/Cas9 system for targeted mutagenesis in sorghum**

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CRISPR and associated protein 9 (CRISPR/Cas9) has been extensively harnessed for genome editing in a wide variety of plant species. Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop across the world and it is also a potential bioenergy resource. With publicly available genomic and genetic information, the progress of agronomic trait improvement in sorghum can move forward rapidly by leveraging genome editing technologies. Genome editing platform in sorghum has been lagging behind other cereal crops due to its low transformation and editing efficiency. Here, we report an efficient sorghum CRISPR/Cas9 system for targeted mutagenesis as delivered through *Agrobacterium* into immature embryos from genotype P898012. Two CRISPR/Cas9 constructs were designed to edit candidate genes underlying quantitative trait loci (QTLs) for flowering time (Sobic.010G045100, *SbFT*) and plant height (Sobic.009G230800, *SbGA2ox5*), respectively. The transformation frequency of this CRISPR/Cas9 system reached 4% for *SbFT* and 8% for *SbGA2ox5*. Both genes were edited in the T<sub>0</sub> generation with editing frequencies of 33% for *SbFT* and 50% for *SbGA2ox5* respectively. The induced mutations were transmissible to T<sub>1</sub> and T<sub>2</sub> generations. Novel mutations at a frequency of 68% for *SbFT* and 24% for *SbGA2ox5* were also observed in T<sub>1</sub> plants from the unedited T<sub>0</sub> plants, which showed the continued activity of Cas9/sgRNA in the following generation if inherited. The plants with null allele mutations for *SbFT* displayed late flowering in both T<sub>1</sub> and T<sub>2</sub> generations. No homozygous mutant plants were obtained for *SbGA2ox5* gene in the T<sub>1</sub> generation, which could be due to potential lethality of biallelic mutation. This study demonstrates a feasible CRISPR/Cas9 system that will facilitate the exploration of functional genomics and crop improvement in sorghum.

Gene / Gene Models described: *SbFT*, *SbGA2ox5*; Sobic.010G045100, Sobic.009G230800

Funding acknowledgement: United States Department of Agriculture (USDA), the Iowa State University Presidential Initiative for Interdisciplinary Research, the Iowa State University Plant Sciences Institute

P84 

## **An intergenic locus *KRN4* controls kernel row number through long-distance regulation of *UNBRANCHED3* expression in maize**

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KERNEL ROW NUMBER4 (*KRN4*) is an intergenic QTL controlling a maize yield trait, and maps close to *UNBRANCHED3* (*UB3*), a negative regulator of *KRN*. However, the mechanism by which *KRN4* controls *UB3* expression remains unknown. In this study, we found that allelic variation in *KRN4* changes *UB3* expression and inflorescence meristem diameter and *KRN* in two sets of near isogenic lines, indicating that *KRN4* is agronomically important. To understand the molecular basis of the interaction between *UB3* and *KRN4*, we used chromatin immunoprecipitation followed by paired-end tag sequencing (ChIA-PET). We also used the short enhancer-like element from different *KRN4* alleles upstream of a minimal CaMV 35S or a *UB3* promoter to drive luciferase expression. Using ChIPseq, we found that both *UB3* and *KRN4* are direct targets of *UB2*, a paralog of *UB3*, and *UB3* expression varies with *UB2*. Two enhancer-binding factors, *OBF1* and *OBF4*, interact with *UB2*, and bind to *KRN4*. Therefore, we propose that *KRN4* regulates *UB3* expression by direct chromatin interactions, and *UB2* may mediate the establishment or maintenance of the appropriate chromatin configuration, and recruits *OBF1* and *OBF4* to form a transcriptional complex to fine tune *UB3* expression and, in turn, *KRN*. These results provide evidence for fine tuning of gene expression by intergenic QTLs in maize, and a new perspective for genetic control of a quantitative trait.

Keywords: Maize (*Zea mays L.*); Kernel row number; Intergenic region; Chromatin interaction; Enhancer.

Gene / Gene Models described: *UB2*, *UB3*, *OBF1*, *OBF4*; Zm00001d031451, Zm00001d052890, Zm00001d030617, Zm00001d012553

Funding acknowledgement: National Science Foundation (NSF)

P85

## **An unexpected link between adult plant resistance and host physiology during interaction of maize with a leaf blight pathogen**

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Adult plant resistance (APR) is a phenomenon in which plants are susceptible to disease as seedlings but resistant at maturity. We recently addressed this trait in maize during interaction with *Cochliobolus carbonum* race 1 (CCR1), a fungal pathogen that causes a severe leaf spot/blight disease. Resistance to CCR1 is conferred by the *Hm1* disease resistance gene, which encodes an NADPH dependent reductase to inactivate HC-toxin, the key virulence effector of CCR1. Plants that lack *hm1* are completely susceptible to CCR1. We were able to generate three partial loss-of-function alleles of *Hm1* by targeted mutagenesis with EMS. All three of these alleles conferred an APR phenotype, the strength of which corresponded exactly with the HC-toxin reductase (HCTR) activity encoded by these alleles. The strength of the APR phenotype was also impacted by the photosynthetic output of the plant, with the longer duration of light transforming APR to seedling resistance and the shorter duration of light completely suppressing resistance, establishing unexpected link between host physiology and resistance. Furthermore, incubation in the dark of green seedlings resulted in complete loss of resistance not only to CCR1 but many other pathogens as well. However, this does not appear to happen to albino seedlings or seedlings that are etiolated. The mechanism for this disconnect is not clear yet but we suspect that some aspect of source-sink relationship between the seed and the seedling may be at work here.

P86 

## Analysis of a cluster of PME pseudogenes at the maize Gametophytic Incompatibility 1 (*Ga1*) locus

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Specialty maize such as organic maize is required to be free from foreign pollen to meet the standards of identity preservation and genetic purity. Inherent reproductive barriers such as the maize cross incompatibility loci, can be utilized to manage the undesirable effects of pollen transfer between GM and organic maize fields. Several gametophytic incompatibility loci have been reported, of which *Ga1*, *Ga2* and *Tcb1* have been well studied. The *Ga1* locus mediates its effect through pollen- and silk-specific functions. Transcriptomic profiling of silks of near isogenic lines of W22 differing at the *Ga1*-locus led to the identification of a candidate gene for the female function. This silk-specific gene, a pectin methylesterase (PME), is designated as *ZmPme3* and we hypothesize it confers exclusion of *gal* pollen. Another gene, a pollen-specific PME designated as *ZmGalP*, was identified by Zhang et. al, as the male factor. Genomic sequence analysis revealed a cluster of PME pseudogenes related to either the silk- or pollen-specific genes at the *gal* locus. A total of 59 full and partial length *ZmPme3*-like sequences spanning a region of 1.1 Mbp were identified at the locus. *ZmGalP*-like pseudogenes are also present in this cluster, interspersed within the *ZmPme3* pseudogenes. The *Tcb1* locus is also on chromosome 4, approximately 44 centimorgans away from *Ga1*. The candidate gene for *Tcb1* is a PME *Pertunda* (Lu et al., 2019), related to *ZmPme3*, but does not appear to lie within a cluster of pseudogenes. Analysis of the *gal* cluster will help to understand the significance of the PME gene family in the context of maize domestication.

Funding acknowledgement: United States Department of Agriculture (USDA)

P87

## C/N partitioning in maize kernels responds to alterations in the mitochondrial respiratory chain

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The importance of C and N partitioning in maize kernels motivated our focus on mutations affecting these features. Using a Mu-seq protocol for sequence-based transposon mapping, we isolated multiple mutants with defective-kernel phenotypes from the UniformMu maize population. Two are presented here. The first is a new “opaque” mutant, identified and confirmed by transgenic complementation to arise from disruption of *RUG3* (*RCC1/UVR8/GEF-like 3*). The *rug3* mutant kernel has a normal starchy endosperm, but a defective vitreous layer. The visible kernel phenotype is severe when grown in spring, but less altered during the autumn planting season in Florida. A primary role of temperature in this phenotypic plasticity is evident when growth is compared under controlled-environment conditions. Analysis of transcripts shows deficient splicing of mitochondrial mRNAs, including those for complex I of the respiratory chain. *RUG3* and other nuclear-encoded splicing factors that target mitochondria, promote folding and subsequent splicing of group II introns from mitochondrial genes. These other factors include PPR (pentatricopeptide repeat) proteins, and maturases. The second mutant was dysfunctional for one of these maturases, *mat1* (nuclear *maturase 1*), and has an empty-pericarp kernel phenotype arising from disruption of the respiratory chain. Although plants have evolved alternative pathways for electron transfer, ATP production relies heavily on complex I. Disruption of complex I and its specific effect on respiration and other mitochondrial functions thus affects the development of maize kernels. Transcriptome profiles from both *mat1* and *rug3* kernels indicate reductions in abundance of mRNAs for biosynthesis of storage proteins and starch. These shifts were consistent with other instances of imbalance in the Opaque2- and PBF1-mediated networks. However, both mutants also show increased abundance of transcripts for constituents of the entire respiratory chain. The latter could represent a compensatory upregulation mediated by ROS or an energy-sensing system.

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P88

## Characterization and cloning of a *semi-dwarf* (*sdw\**) mutant affecting plant architecture in maize

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Plant architecture traits such as leaf angle, plant height and inflorescence branching are key determinants of grain yield in maize, yet the underlying genetic mechanisms regulating these traits are complex and incompletely understood. We identified and characterized a recessive, Mo17\*EMS-derived maize mutant, *sdw\** (for *semi-dwarf*), that displays reduced plant height, small leaves and has reduced tassel-branch number. Mutant plants have short leaf sheaths and short leaf blades when compared to normal siblings. The semi-dwarf stature of the mutants is due to compressed internodes and not reduced internode numbers. We used the maize SNP50 array to perform bulked-segregant analysis (BSA) on an F<sub>2</sub> mapping population to localize a peak of mutant-linked markers to a 10 MB region at the end of chromosome 3S. We confirmed the peak limits by fine mapping and then used BSA-seq to align reads from the mutant pool to both the B73 and Mo17 genomes and call non-Mo17 SNPs. Within the 0.5MB fine-map interval, there was one gene model that contained a G>A SNP predicted to result in a causative mutation. The gene encodes 3-epi-6-deoxocathasterone 23-monooxygenase, a P450 reported to be involved in brassinosteroid biosynthesis. The mutation changes a highly conserved G to D, potentially altering gene function. A second allele from the Mu-illumina project was identified by its similar phenotype and failure to complement the original *sdw\** isolate. Mutants display defects in mesocotyl and shoot internode elongation when germinated in the dark; this constitutive photomorphogenic phenotype in the dark is shared by other BR biosynthetic mutants in Arabidopsis and rice. These defects are mimicked by wild-type seeds germinating in the presence of the BR biosynthesis inhibitor (PCZ). Based on these results, we propose that *sdw\** is a BR biosynthetic gene and plays a role in plant growth and development.

Funding acknowledgement: National Science Foundation (NSF)

P89 

## Characterization and genetic mapping of the *carbohydrate partitioning defective60* mutant in maize

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Carbohydrate partitioning is the process by which sugars, primarily sucrose, synthesized in the photosynthetic source tissues (mature leaves) are mobilized to non-photosynthetic (sink) tissues, such as roots, seeds, and developing organs. To identify genes controlling carbohydrate partitioning, we identified a number of mutants, termed the *carbohydrate partitioning defective* (*cpd*) mutants, which overaccumulate starch and sugars within their leaves. One such recessive mutant, *cpd60*, hyperaccumulates starch and sugars, such as, sucrose, glucose, and fructose in its leaves, and displays stunted growth, reduced fertility, leaf chlorosis, and accumulation of anthocyanins in the mature leaves. Furthermore, ectopic lignin depositions were observed in the phloem tissues of mature mutant leaves. The mutation responsible for the *cpd60* phenotype has been mapped to the lower arm of Chromosome 1 by Bulk Segregant Analysis (BSA). By using polymorphic markers, we have fine mapped the causative mutation to a 600 kb region, which is predicted to contain nine coding sequences. Whole genome sequencing approaches are underway to identify the causative mutation responsible for the *cpd60* phenotype. Furthermore, three more alleles of *cpd60* have been identified and are being characterized to elucidate the gene function.

The identification of the gene responsible for the *cpd60* phenotype will provide new insights into the genetic regulation of sugar metabolism and allocation in maize. With this knowledge, we can translate our understanding of carbohydrate partitioning to other crop species, such as, sorghum and sugarcane, for genetic improvements to increase food yield and biofuel production.

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



P90

## Characterization and mapping of a maize mutant affecting endosperm gene networks

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In cereal crops, a major proportion of grain weight is determined by the endosperm that represents a unique tissue with highly organized cellular structures. This storage structure is the result of a second fertilization and supports embryo development and seedling germination. *spk*\*N600C is a single-gene recessive maize mutant generated by EMS mutagenesis. Homozygotes show incomplete anthocyanin pigmentation in the aleurone, floury endosperm and reduced seed weight. Embryos do not develop properly and fail to germinate. Quantitative PCR shows that this mutant has reduced expression of *nkd1* and *nkd2* TFs that play a central role in regulating maize endosperm. To map this mutant, we are using BSR-Seq approach which provides a high resolution map with SNP markers that will identify the chromosomal location of the causal locus. Wildtype and mutant pools were created from the mapping population and subsequent RNA-seq was carried out which we are currently processing for mapping and expression study. Because this gene is critical for grain development, quality and yield, identifying the gene and studying its molecular function will give a better understanding which could be directly or indirectly applicable for genetics and breeding.

Funding acknowledgement: National Science Foundation (NSF)

P91

## Characterization of a mutant of *Setaria viridis* (green foxtail) with delayed flowering under short-day conditions

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Photoperiodic sensitivity is a major agronomic trait that determines vegetative and reproductive growth, and is thus particularly important for crop yield and quality. The fast-cycling *Setaria viridis* has been proposed as a model grass for functional genomic studies in food and bioenergy panicoid crops, including maize and sorghum. A N-nitroso-N-methylurea (NMU) mutagenesis of *S. viridis* was conducted and the population screened for photoperiodic mutant phenotypes. Among approximately 2,700 screened M2 families, we identified one recessive photoperiod-insensitive mutant, which showed late flowering and increased panicle length under short-day (SD) conditions (12:12 light:dark), while the morphology and phenotype were not affected under long-day (LD) conditions (16:8 light:dark). Bulked segregant analysis mapped a quantitative trait loci (QTL) peak to chromosome 4, within an approximately 24 Mb wide interval. Within this wide peak, there were only five nonsynonymous single nucleotide polymorphisms (SNPs). Among them, a candidate gene (*SvCOI*) was found that was homologous to *CONSTANS* (*CO*) in *Arabidopsis thaliana*. A DNA sequencing analysis identified a single nucleotide mutation in the second exon of *SvCOI*, which led to an Arg-to-Trp substitution in CCT (*CO*, *CO*-like and *TOC1*) motif, predicted to be deleterious to protein function. qPCR results show little difference in gene expression of the *CO* homolog between SD and LD conditions, but marked down-stream effects, suggesting that the *CO* protein is expressed but impaired in function. This study provides novel insights into the roles of *CONSTANS* in regulating photoperiodic sensing in panicoid grass.

Funding acknowledgement: National Science Foundation (NSF)



P92

## Characterization of novel transposon and CRISPR-Cas9 gene edited maize *brachytic 2* alleles

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Maize plants with mutations at *brachytic 2* (*br2*) reduce plant height through internode shortening while maintaining the rest of the plant's relative size. The gene product of *Br2* encodes an ATP binding cassette type B (ABCB) auxin transporter. Several *br2* mutations have been previously reported, notably independent characterizations of 8 bp and 241 bp deletions (*br2-23* and *br2-qpa1*, respectively), a single missense mutation (*br2-qph1*), and a MITE transposon insertion (*br2-NC238*). Two new *br2* mutations (designated *br2-7081* and *br2-7861*) have now been characterized with different genetic mechanisms than prior reports providing further examples of functional genetic variation in maize. A gene edited *br2* allele (designated *br2-1005*) has also been generated through use of CRISPR-Cas9 technology. Both novel *br2* transposon mutants in this report (*br2-7081*, *br2-7861*) arose spontaneously, independently and were identified in proprietary maize germplasm; both contain insertions that result in frameshifts with presence of premature stop codons. The *br2-7081* allele contains a 4.7 kb insertion in exon 5, which is identified as a Ty1-copia family long terminal repeat (LTR) retrotransposon. The *br2-7861* allele contains a 579 bp insertion in intron 4, which is identified as a partial Sirevirus LTR retrotransposon, and encodes a transcribed exon of 190 bp. A gene edit in *br2* (*br2-1005*) was generated when a CRISPR-Cas9-induced double strand break was repaired by the plant via non-homologous end joining, causing a 1 bp frameshift resulting in a premature stop codon in exon 5. The novel mutant alleles reported here provide further examples of functional genetic variation in maize *br2*, either through inherent genetic variation mechanisms or generated using site-directed nuclease technology. The *br2-1005* allele highlights the utility of gene editing to phenocopy naturally occurring mutations, and all three alleles in this report provide additional opportunities to research the brachytic semi-dwarf phenotype in maize.

Gene / Gene Models described: *br2*; GRMZM2G315375\_T01

P93 

## Characterization of the maize hypersensitive defense response

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The hypersensitive defense response (HR) is defined as a rapid, localized host cell death at the point of pathogen ingress. It is often a very effective defense strategy and has been observed in all higher plants. Initiation of HR is controlled by dominant resistance (R) genes which encode proteins that are activated upon the detection of specific pathogen-derived molecules. Rp1-D21 is an aberrant R-protein that activates HR spontaneously in the absence of the cognate pathogen. Using a variety of genetics, quantitative genetics, molecular biology and genomics approaches, we have characterized a set of genes and mechanisms that mediate the strength of the HR conferred by Rp1-D21. These include, intra- and inter-molecular interactions, reactive oxygen species produced in the mitochondria, lignin biosynthesis and the ubiquitin-mediated protein degradation pathway. We will present some of our recent results.

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

P94

## Characterization of the novel maize *carbohydrate partitioning defective* mutant *P135-21B*

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Sugar synthesized by photosynthesis needs to be efficiently exported from the leaves as sucrose to feed developing sinks. There are a class of maize mutants called *carbohydrate partitioning defective* (*cpd*) mutants which overaccumulate starch and soluble sugars in their leaves. High sugar levels in the leaves result in a characteristic phenotype, including repression of photosynthetic gene expression and chlorosis and anthocyanin accumulation in leaves. *P135-21B*, a novel maize mutant conditioned by a semi-dominant mutation, exhibits a progressive basipetal chlorosis and starch accumulation. Three main questions were addressed: whether both starch and soluble sugars hyperaccumulate, why there is carbohydrate hyperaccumulation in mutant leaves, and what is the causal gene. In order to locate and quantify the carbohydrate accumulation in source leaves, an Iodine/Potassium Iodide stain and a quantitative measurement of sugar and starch levels using High Pressure Anion Exchange Chromatography (HPAEC) were performed. Aniline Blue staining of adult leaves suggested that the mutant phenotype may be caused by a partial blockage in the phloem due to hyperaccumulation of callose. To confirm the initial results and determine whether callose accumulation precedes carbohydrate buildup, the Aniline Blue stain will be repeated on both adult and developing leaves. *P135-21B* segregates 3:1 in the B73 inbred background and is likely conditioned by two independent loci. To find the rough mapping interval, pools of mutants and wild type siblings were collected and DNA was extracted for a bulked segregant analysis. A 5 MB region on chromosome 1S and a similar interval on chromosome 7S were found to be enriched in the mutants and deficient in the wild type siblings. In order to find the causal genes, recombination breakpoints are being screened with polymorphic markers to narrow this interval down. Neither locus is shared with any previously characterized *cpd* mutant; thus, *P135-21B* is a novel gene.

Funding acknowledgement: National Science Foundation (NSF)

P95 

## Characterizing the cytokinin responsive determinants of leaf patterning in maize

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Spatially organized patterns of cells and tissues are one of the driving forces behind normal organ formation. A complete understanding of the determinants that regulate how cells become patterned has not been achieved. We use the maize leaf as a model to study patterning determinants, because its component tissues are organized in a distinct proximal-distal (P-D) pattern. Previous analyses of the semi-dominant *Hairy Sheath Frayed1* (*Hsfl*) mutant revealed that altered cytokinin (CK) signaling can influence P-D leaf patterning. *Hsfl* mutants have ectopic outgrowths on the distal blade, termed “prongs”, that consist of proximal tissues. To understand how CK signaling drives prong formation, laser-capture microdissection (LCM) coupled with whole transcriptome sequencing (RNA-Seq) was used to identify approximately 800 differentially expressed (DE) genes. Enriched among these DE genes are transcription factors (TFs) associated with organ formation. Epistatic analyses with mutants of the DE genes *delayed flower1* (*dlf1*), *liguleless3* (*lg3*), and *tassels replace upper1* (*tru1*) will be reported. Expression of a subset of the DE genes at different stages of prong development will also be presented. A genetic modifier was discovered in the A619 inbred line, which enhances the *Hsfl* phenotype. Segregation models were tested and the enhancer appears to segregate as a single recessive locus.

Funding acknowledgement: National Science Foundation (NSF)

P96

## Characterizing the role of the duplicated genes *Pho1;2a* and *Pho1;2b* in maize phosphorus homeostasis

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Maize is a good model for the study of gene duplication, genetics, domestication and evolution. In the recent evolutionary past, maize experienced whole genome duplication and subsequent gene loss through the process of fractionation. Although in the majority of cases only a single copy remains from each pair of paralogs that resulted duplication, in a substantial number of cases both paralogs have been retained. One such pair consists of the genes *Pho1;2a* and *Pho1;2b*, co-orthologs of the major *Arabidopsis* phosphate translocator/sensor *Pho1*. We are looking to test whether *Pho1;2a* and *Pho1;2b* have a non-redundant functions, and whether they exhibit subfunctionalization with respect to the single-copy *Pho1;2* genes of related species, such as sorghum (*Sorghum bicolor*) or rice (*Oryza sativa*). In *Oryza sativa*, the single *Pho1;2* gene is associated with a *cis*-Natural Antisense Transcript, which acts to enhance *Pho1;2* polysome association and translation under low phosphorus conditions. We will present evidence that the maize *Pho1;2a* and *Pho1;2b* genes have diverged with respect to the accumulation of sense and antisense transcripts.

Gene / Gene Models described: *Pho1;2a*, *Pho1;2b*, *Pho1;2*; GRMZM2G466545, GRMZM2G058444, Os02t0809800-01

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P97

## Cloning genes of maize *defective kernel* mutants

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In the 1970s, Neuffer and Sheridan generated a chemically induced *defective kernel* (*dek*) mutant collection with the potential to uncover critical genes for seed development. To locate such mutations with next generation sequencing, the *dek* phenotypes were introgressed into two inbred lines to take advantage of maize haplotype variations and their sequenced genomes. Although bulked segregant analysis (BSA) is widely used in *Arabidopsis* and rice to identify existing or induced variants that are linked to phenotypes, it remains challenging for crops with large genomes, such as maize. Here, we identified several genes of maize *defective kernel* mutants with newly developed user-friendly analysis pipelines, which will take fastq files derived from nextGen paired-end DNA and cDNA sequencing as input, call on several well established and freely available genomic analysis tools to call SNPs and INDELS, and generate lists of the most likely causal mutations together with variant index plots to locate the mutation to a specific sequence position on a chromosome. The pipelines were validated with a known strawberry mutation before cloning the *dek* mutants, thereby enabling phenotypic analysis of large genomes by next-generation sequencing.

Funding acknowledgement: Selman Waksman Chair in Molecular Genetics

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## Connecting genomic variation to yield-related traits through maize kernel development proteome

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Exploring the link between gene function, protein action, and agronomic traits is an important goal of plant genetics. Proteome-based genome-wide association studies (pGWAS) could provide evidence of the relationship between protein composition and traits, but technological limitations remain a challenge. In this study, a pGWAS in maize was performed to explore the genetics of the kernel proteome and to connect genomic variation to proteins involved in yield-related traits. In total, 473 proteins involved in cellular and metabolic processes corresponding to 286 unigenes were quantified by mass spectrometry in maize kernels 20 days after pollination. 28 significant pQTLs were identified, including 19 trans-pQTLs and 9 cis-pQTLs. These 28 pQTLs accounted for the abundance changes of 25 unique proteins and overlapped with 20 eQTLs, nine metabolic QTLs, and with 15 QTLs of yield-related traits identified in different genetic background by genetic association and linkage mapping. Furthermore, pQTLs involved in yield-related pathways are highlighted, including starch and caloric content, amino acid metabolism, and hormone regulation pathways. Together, the present study provides a useful reference for the integration of -omics and yield-related trait gene mining, and could help improve maize yield and seed quality.

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P99

## Conservation of promoter architecture preference during auxin-mediated transcription

(submitted by Mallorie Taylor-Teeple <[mmtt@uw.edu](mailto:mmtt@uw.edu)>)

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The hormone auxin is involved in nearly every aspect of plant growth and development. How a single molecule gives rise to so many different downstream responses has been a long-standing question in the field of plant biology. One hypothesis proposes that interactions between different auxin response transcription factors (ARFs) and the promoters of auxin-responsive genes can lead to unique transcriptional responses. Previously, we expressed components of the *Arabidopsis thaliana* auxin response pathway in yeast to measure transcriptional activation on promoter variants by different ARFs using flow cytometry. We have found that changing the number, sequence, spacing, or orientation of auxin response elements (AuxREs) in a promoter sequence affects transcription in predictable ways. However, contrary to our expectations, different *Arabidopsis* ARFs all seem to share a preference for the same AuxREs. Maize ARFs are now being expressed in our yeast synthetic system to determine if this relationship is conserved across species. Results from our synthetic approach, along with recently published DAP-seq binding data in maize (Galli et al., 2018), suggests that auxin response specificity may be determined by some factor other than individual ARF binding site selection.

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P100

## CRISPR/Cas9 gene knockout in arabidopsis plants

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Genetic engineering is an important field in biology. Being able to modify genomes provides the opportunity to produce genetically modified crops, create animal models for studying human diseases, or to investigate the basic mechanisms of plant and animal biology. Various technologies have been developed, but CRISPR (Clustered regularly-interspaced short palindromic repeats)/Cas9 has proven to be an efficient and precise mechanism for editing genomes through targeted mutagenesis (Schiml, Fauser, & Puchta, 2014). Using an enzyme called Cas9 and guide RNA (gRNA), scientists can target a specific region in the genome and make a double stranded break so that any DNA can then be added or removed through DNA repair mechanisms (Jiang, Yang, & Weeks, 2014). Our project aimed at designing and implementing CRISPR/Cas9 technology on a model organism *Arabidopsis thaliana* (rockcrest) to analyze the function of plant genes involved in cold tolerance. We selected six such genes (from the website database [arabidopsis.org](http://arabidopsis.org)) and, using published CRISPR/Cas9 protocols (Čermák, et al., 2017), selected appropriate gRNA regions to create approximately 200 bp out of frame deletions in coding parts of the genes and constructed transformation vectors, using golden gate cloning technology. The vectors at each of the cloning steps were analyzed by restriction digests, colony PCR, and sequencing, demonstrating the success of vector assembly. The T0 plants were transformed with T-DNA transformation vectors for all selected genes and seedlings were screened for correct transformations. The screening process included DNA extraction and purification, PCR, and sequencing. Protocols we implemented will be used to further investigate the function of *Arabidopsis* genes involved in cold stress response.

Funding acknowledgement: National Science Foundation (NSF)

P101



## Cytokinin promotes jasmonic acid accumulation in growing leaves

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The maize leaf is an excellent model to better understand the fundamental signals regulating plant growth, since the two cellular processes driving growth – division and elongation – are spatially separated into distinct zones at the leaf base. Our analysis of the semi-dominant *Hairy Sheath Frayed1 (Hsf1)* mutant indicated it had reduced leaf growth due to hypersignaling of the phytohormone cytokinin (CK). CK typically functions to promote cell proliferation but can also repress growth in certain contexts; although how repression is mediated is not well-defined. Our analysis of *Hsf1* revealed that it over accumulates jasmonic acid (JA) in growing leaves, a hormone previously shown to both repress cell division and activate defense pathways. To investigate this novel connection, we determined that exogenous JA application on inbred B73 repressed leaf growth, while in the JA-deficient *tasselseed1 (ts1)* mutant and *opr7opr8* double mutant leaf growth is enhanced. We assessed JA pathway gene expression levels in the division and elongation zones of emerging leaves of *Hsf1/+* and wild type (WT) seedlings. Several JA biosynthesis and responsive genes were significantly upregulated in the growth zone of *Hsf1* mutants compared to WT sibs. Overall, our results indicate CK signaling promotes JA accumulation through up-regulation of JA biosynthesis. Further analysis of this new example of hormone crosstalk may provide insights into the mechanisms by which plants balance growth with other processes, such as defense response.

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

P102 

## Detection of genetic sequences in maize using dextrin-capped gold nanoparticles

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Gold nanoparticles (AuNPs) are one of several DNA detection methods that have been employed in diagnostic and bio-sensory assays ranging from cancer detection in hospitals, to virus and pathogen detection in the field. AuNPs have been extensively used because of their stability, and controlled geometrical, visual, and surface chemical properties. This study investigates the use of AuNPs as a detection assay for DNA sequences in maize. In this assay, we use dextrin-capped gold nanoparticles (d-AuNPs) for an unamplified genomic DNA biosensor. The aggregation and dispersion characteristics of the d-AuNPs in an ionic salt environment are utilized for this sequence specific detection assay. The d-AuNPs form a complex between the single-stranded DNA probe (ssDNAp), the target DNA, and the nanoparticles to achieve stability. This stability causes a color display of red/pink when target DNA is present, but when there is no target DNA, a blue/purple color is displayed. Through further research and application, we hope to use this assay to assist breeders in their selection process with a rapid simple method of detection of native sequences, transgenic insertions, introgressed regions, and recurrent parent DNA.

P103 

## Development of an amenable system for site-specific addition to a maize chromosome

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Currently, transgenic maize is produced by random integration of a transgenes into the plant. This works for single genes, but not as well for multiple traits. Identifying plants that contain several transgenes becomes a very difficult task. Gene stacking at a single location in the genome would make combining multiple transgenes into plants a simpler process. This presentation focuses on the development of a system that allows for transgenes to be sequentially added to a specific site in the maize genome. The system utilizes two recombinases, Cre recombinase and  $\phi$ C31 Integrase, to remove a selectable marker and to integrate transgenes. An initial construct containing a selectable marker, flanked by LoxP sites, which are acted upon by Cre recombinase, and an attP site, were transformed. The selectable marker was then removed from the integrated transgene by exposure to Cre recombinase. Two amendment constructs would enable modification of the integrated construct by utilizing complementary attP and attB sites, which are acted upon by  $\phi$ C31 Integrase. The amendment constructs contain cargo and a promoterless selectable marker which, upon successful recombination with the target site, would restore expression of the selectable marker. Successful demonstration of this system would simplify generation of multi-transgene plants, and the assembly of multi-gene pathways in plants. Funded by NSF IOS-1339198

Funding acknowledgement: National Science Foundation (NSF)



P104

### Development of an iPCR method to identify genes mutated by transposed *activator* in maize

(submitted by Namrata Maharjan <[Namrata.Maharjan@sdsstate.edu](mailto:Namrata.Maharjan@sdsstate.edu)>)

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We have several mutations of interest that appear to be induced by the insertion of a transposed *Activator* element (*trAc*). We present a technique to determine the sequences of regions that flank a gene mutated by a *trAc* that uses the known DNA sequence of *Ac*. Inverse polymerase chain reaction (iPCR) is a technique where forward and reverse primers are developed for a known sequence, in this case *Ac*, but are oriented opposite to each other. The PCR amplification for flanking sequence is then accomplished by fragmenting the template DNA using a restriction enzyme and then circularizing the DNA fragments using T4 ligase. It is expected that that primers now oriented toward each other on the fragment of interest. To validate this protocol, we tested a known DNA sequence of *bz1-m2(Ac)* from NCBI (Accession no AF355378.1). Because of the potential presence of cryptic *Ac*s, the DNA was digested with the methyl sensitive restriction enzyme HhaI to produce a fragment near the 5' end of the *Ac* as well as at other unknown locations. The fragments were then ligated. The *Ac* specific primers were used to amplify ligated restriction fragments that possessed *Ac*. In this test one band was produced on an agarose gel. The resulting band was excised and sequenced. The results show a 99% sequence match with the original sequence from NCBI, including regions of *bz1* and *Ac*. These results have encouraged us to use this technique to identify flanking sequences of unknown mutations that have been putatively induced by the insertion of *Ac*.

Funding acknowledgement: National Science Foundation (NSF)

P105

### Developmental roles of genes in B-vitamin biosynthesis in maize

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The B-vitamins are essential co-factors for central metabolism in all organisms. In plants, B-vitamins have surprising emerging roles in development, stress tolerance and pathogen resistance. Hence, there is a paramount interest in understanding the regulation of vitamin biosynthesis as well as the consequences of vitamin deficiency in crop species. To facilitate genetic analysis of B-vitamin biosynthesis and functions in maize, we have mined the UniformMu transposon resource to identify insertional mutations in vitamin pathway genes. A screen of 192 insertion lines for seed and seedling phenotypes identified mutations in biotin, pyridoxine and NAD biosynthetic pathways. Because B-vitamins are essential for survival, a subset of null mutations have seed lethal phenotypes that prevent elucidation of more subtle, but physiologically important, metabolic responses to sub-optimal vitamin status (functional deficiency). As a means to surmount this barrier, we demonstrate a strategy for genetic fine-tuning of vitamin status based on construction of heterozygotes that combine strong and hypomorphic mutant alleles to produce plants with graded vitamin deficiency. Similarly, use of a hypomorphic *bio1* allele enabled analysis of transcriptome and metabolome responses to incipient biotin deficiency in seedling leaves. We show that pipelicolic acid accumulation is early metabolic response to sub-optimal biotin status revealing an intriguing connection between biotin status, lysine metabolism and systemic disease resistance signaling. Overall, both strong and hypomorphic alleles of mutants in UniformMu population are useful genetic resources to investigate gene functions quantitatively as well as to facilitate genetic analyses of mutants generated by other methods such as EMS and genome editing.

Gene / Gene Models described: ; GRMZM2G102156

Funding acknowledgement: National Science Foundation (NSF)



P106 

## **Discovery and characterization of mutants defective in gravitropic growth of shoots**

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Immediately following germination, plants reorient root and shoot growth response to gravity to effectively explore soil and obtain light, respectively. To better understand how this process is regulated we carried out a genetic screen to identify mutants that either failed to, or were slow to, reorient in response to a gravity vector in the dark. Using family-wise screening of Next-Generation EMS Mutagenized families, 30 mutants were identified that repeatedly exhibited slow shoot turnaround out of 800 tested families. Among these, 8 exhibited short thick shoots (sts) in the dark. Remarkably, these all display attributes consistent with in Brassinosteroid (BR) deficiency. Brassinosteroids are growth promoting phytohormones controlling cell elongation and multiple developmental processes. Similar to the known BR-deficient mutants (na1, na2, lil/brd1) these mutants exhibited persistence of pistils in the tassel florets (POPIT), upright leaves, and short stature similar to BR-deficient mutants. All 30 slow shoot turnaround families were grown to maturity and screened for architectural abnormalities with a focus on dwarfism and antigravitropism. No mutants with phenotypes similar to lazy plant1 were recovered in this screen. All known BR-deficient mutants of maize were germinated in the dark and tested for gravitropic growth. Although dwarfism was apparent in na1 and na2 lines, seedlings did not display the short thick shoots or slow shoot turnaround that characterized the sts mutants. Unlike these BR-deficient mutants, brd1 seedlings defective in BR-6-oxidase, did exhibit short, thick shoots and slow shoot turnaround when inverted in the dark. It is possible that the 8 sts mutants are new alleles of brd1 or are alleles in other steps of BR synthesis or signal transduction.

Funding acknowledgement: National Science Foundation (NSF)

P107

**Discovery of a novel 9-allene oxide cyclase enzyme in maize defines a key biosynthetic branch point between jasmonate and death acid signals**

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In plants jasmonate hormones orchestrate diverse physiological roles in reproductive development and inducible defenses that protect against biotic agents. Jasmonates are cyclopentanone oxylipins derived from the dioxygenation of linolenic acid (18:3) precursors by lipoxygenases (LOX) acting with regiospecificity at carbon 13 (13-LOX) followed by the sequential action of 13-allene oxide synthases (13-AOS) and 13-allene oxide cyclases (13-AOC) to form 12-oxo-phytodienoic acid (12-OPDA). LOX enzymes acting on carbon 9 (9-LOX) also generate oxylipins including cyclopente(a)nonenes functioning in stress protection. In maize, we previously described a series of linoleic (18:2) and 18:3 derived 9-LOX cyclopente(a)none death acids as positional isomers of the jasmonate pathway displaying broad transcriptional and cytotoxic activities. Unlike the defined jasmonate pathway, how parallel 9-LOX-derived cyclopentenones such as 10-oxo-11-phytodienoic acid (10-OPDA) and 10-oxo-11-phytoenoic acid (10-OPEA) are enzymatically produced remained both a mystery and research obstacle. Using a forward genetics approach, we employed metabolite-based Genome-Wide Association Studies (mGWAS) and linkage analyses in biparental populations to uncover a significant shared locus on chromosome 6 that drives death acid production. Informed by transcriptomic analyses, the top gene candidate was part of a large conserved enzyme family associated with diverse catalysis and plant stress protection. Enzyme assays using *Agrobacterium*-mediated heterologous expression in *Nicotiana benthamiana* confirmed that co-expression of a 9-LOX (ZmLOX5) and a 9,13 dual specific AOS (ZmAOS1) with the candidate gene yields highly significant and substrate specific production of both 10-OPEA and 10-OPDA. Similar to strict enzymatic control present in jasmonate biosynthesis, our results demonstrate discovery of specific 9-allene oxide cyclase (9-AOC) enzyme in plants mediating the controlled biosynthesis of death acids. Unlike the complex, redundant and often dual-specific activities of LOX and AOS family enzymes, the identification of genes encoding 9-AOC branch point enzymes now enable mutational studies to examine specific physiological roles for death acids in signaling and stress protection.

Gene / Gene Models described: *LOX5*, *AOS1*; GRMZM2G102760, GRMZM2G067225

Funding acknowledgement: National Science Foundation (NSF)

P108

## **Drought stress prior to inoculation promotes maize resistance against *Cochliobolus heterostrophus***

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In nature, plants are simultaneously challenged by multiple forms of biotic and abiotic stress that combine for diverse effects on crop production. Here we examined disease resistance under drought stress conditions and observed that the magnitude of fungal-elicited maize responses are quantitatively dependent on the duration of drought prior to inoculation (DPI). Comparative analysis of watered and drought stressed *Cochliobolus heterostrophus*-infected plants using metabolomic fingerprinting resulted in complete multivariate separation of the two global metabolomes with 2,367 significant molecular features. Among these features, the drought responsive oxylipin 12-oxo-phytodienoic acid (12-OPDA) was strongly elicited by *C. heterostrophus* infection in plants undergoing DPI and displayed a significant negative relationship with fungal growth. The phytohormone abscisic acid predictably increased in response to drought stress but was curiously suppressed in *C. heterostrophus*-infected tissues vs. damaged controls. Examination of ent-kaurene-,  $\beta$ -macrocarpene- and benzoxazinoid-related antibiotics in fungal-elicited tissues displayed drought-dependent increases in production over an eight-day time course. Transcript accumulation of the 1,3- $\beta$ -glucanase PR6mb, pathogenesis-related 4 (PR4), and chitinase genes also demonstrated positive relationships between pathogen elicitation and the duration of DPI. Collectively, our results indicate that drought stress potentiates maize defense mechanisms, leading to heightened resistance against the common maize pathogen *C. heterostrophus*.

Funding acknowledgement: United States Department of Agriculture (USDA)

P109 

## **Ectopic expression of a heterologous glutaredoxin enhances tolerance to multiple abiotic stressors and grain yield in field grown maize**

(submitted by Tej Man Tamang <[tejman@ksu.edu](mailto:tejman@ksu.edu)>)

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Abiotic stress (heat and drought) is an important constraint to corn production, particularly during reproductive stage, which is the most sensitive and critical for seed set and grain yield. Ectopic expression of the *Arabidopsis thaliana* glutaredoxin S17 (AtGRXS17) gene in tomato resulted in plants with higher heat and drought tolerance than wild-type plants during vegetative growth. Here, we report that ectopic expression of AtGRXS17 in maize also enhanced tolerance to drought and heat stress during reproductive stages under field conditions. In field tests, AtGRXS17-expressing maize events displayed higher kernel set, resulting in a 6-fold increase in yield in comparison to the non-transgenic counterparts when challenged with heat stress at tasseling through grain-filling. Similarly, AtGRXS17-expressing plants yielded 2-fold and 1.5-fold more grain weight per plant than wild-type when challenged with drought stress field conditions at tasseling stage and silking stage, respectively. Our results present a robust and simple strategy for meeting rising yield demands in maize.

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

P110

### Effects of root exudates from three *Zea mays* inbred lines on the growth of two rhizosphere microbes, *M. luteus* and *E. coli*

(submitted by Davron Hanley <[davron.hanley@doane.edu](mailto:davron.hanley@doane.edu)>)

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The rhizosphere soil and root tissue is home for a wide array of microbial species. Many of these microorganisms such as *Micrococcus* species and *Escherichia coli* have been observed to form strong symbiotic relationships with the roots of *Zea mays*. It is theorised that these relationships are driven by compounds in the plant's root exudate that promote microbial growth. Root exudates provide a mechanism by which plants can manipulate the microbial community within the rhizosphere, thereby attracting microorganisms which protect it from infection and stress and promoting production of nutrients in the soil that are essential to plant growth. The chemical composition of root exudates differs between genotypes of *Zea mays*. Identifying chemicals in root exudate which make this relationship stronger and more efficient can lead to varieties of corn that rely less on soil amendments such as fertilizer. The aim of this project is to determine how the exudates of genotypes of *Zea mays* compare in their ability to recruit beneficial soil microbes. *E. coli* and *M. luteus* were grown in low nutrient liquid cultures containing root exudate extracted from three different genotypes all known to have different root exudate composition; CML103, B97 and B73. The growth of both bacterial species in each genotype of root exudate was quantified using a hemocytometer and image analysis.

Funding acknowledgement: National Science Foundation (NSF)

P111 

### EMS mutagenesis of MM501D

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Ethylmethanesulfonate (EMS) is a widely used chemical mutagen that typically produces heritable C-T or G-A mutations when applied at a range of concentrations to the germline or meristematic cells of an organism. We used a modified version of a Neuffer et al. (2009) protocol to create M2 mutant populations in MM501D. We delivered EMS in paraffin oil to pollen at a dose of 100ul EMS in 150mL oil, mixed with approximately 15mL of freshly collected pollen. Each batch was used to pollinate approximately 100 ears. Seed set ranged from 100 to 1900 seeds per treatment batch with an average of 7 seeds per ear. In total 4,953 M1 seeds were produced, but due to germination, sterility and plant health, we were only able to recover M2 populations from 3,949 M1 plants. Segregating kernel mutations observed on M2 ears ranged from 1-23% of ears per treatment batch. We present a resource with the potential to explore external partnerships.

P112

## **Engineering nitrogen symbiosis for Africa (ENSA): Towards nodulating maize** (submitted by Doris Albinsky <[da436@cam.ac.uk](mailto:da436@cam.ac.uk)>)

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Within the ENSA (Engineering Nitrogen Symbiosis for Africa) project we are aiming at establishing a sustainable, efficient nitrogen fixing symbiosis in maize, primarily to help small-holder farmers in Sub-Saharan Africa to become independent of nitrogen fertilisers.

It is widely accepted that the nitrogen fixing root-nodule symbiosis (RNS) in legumes evolved from the more ancient arbuscular mycorrhizal symbiosis (AMS), which is found at present in approx. 80% of all land plants. Hence, commonalities in the signaling events (Common Signaling Pathway; CSP) initiated by the perception of rhizobial Nod-factors in RNS and fungal Myc-factors in AMS between legumes and cereals, foremost rice, were unveiled, paving the way for the genetic engineering of nitrogen fixation in maize.

On the grounds of AM phenotyping, we present data demonstrating the functional conservation of known CSP proteins in maize as well as revealing deviations in the perception of fungal signals in maize, rice and legumes.

Funding acknowledgement: Bill & Melinda Gates Foundation

P113 

## **Evaluating the maize B chromosome for trait deployment**

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B chromosomes are non-essential chromosomes found in many plant and animal species, including maize. Non-Mendelian inheritance is a common property of B chromosomes and in maize the B chromosome is known to undergo non-disjunction at very high rates, primarily during the second pollen mitosis. The maize B chromosome has been proposed as a platform for expressing useful traits in maize, and while the maize B chromosome has been studied for over a century, we have only recently begun to understand or evaluate its potential for agriculture. To this end, we demonstrate several useful properties of the B chromosome and discuss several challenges with broad acre deployment of traits expressed from a B chromosome.

P114 

## Evolutionary conservation of protein-protein interaction involved in the splicing of U12-type introns

(submitted by Laurel Levine <[lalevine@oakland.edu](mailto:lalevine@oakland.edu)>)

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The vast majority of eukaryotic genomes contain a rare group of introns called minor or U12-type introns. Comprising approximately 0.5% of total introns, these are spliced by a separate minor spliceosome. The mechanism of U12-type intron-dependent splicing and its biological relevance is not well understood. However, growing reports of mutations that interfere with the splicing of U12-type introns have been linked to developmental defects in both plants and animals. We recently reported a maize mutant of a novel RNA Binding Motif Protein 48 (RBM48). The *rbm48* mutant exhibits severe developmental defects in endosperm and genome-wide aberration of U12-type intron splicing (Bai et al., 2019). Using CRISPR/Cas9-mediated functional knockout of human orthologous RBM48 gene in a cancer cell line, we recently demonstrated the function of RBM48 in U12-type intron splicing is evolutionarily conserved between maize and humans (data not presented). Additionally, human RBM48 was reported to interact with an Armadillo Repeat Containing Protein 7 (ARMC7) of unknown function (Hart et al., 2015). ARMC7 is an essential gene in humans and shares an ortholog in maize. To investigate if maize RBM48 also interacts with ARMC7, we recombinantly expressed maize GST-RBM48 and ARMC7-His in *E. coli*, whose lysates were used in an *in vitro* pull-down based on GST tag immobilization. Interaction was detected through the use of SDS-PAGE and a His-specific antibody. Our data show a clear interaction between ARMC7 and RBM48. Using a similar *in vitro* protein pull down assay, we demonstrate that ARMC7 also interacts with another maize U12-type splicing factor, RGH3 (Rough Endosperm 3). In humans, the RGH3 homolog ZRSR2 is also required for the proper splicing of U12-type introns. Our data suggests the fundamental mechanism of U12-type intron splicing is conserved between maize and humans.

Funding acknowledgement: National Science Foundation (NSF)

P115 

## Expanding resources for maize genomics research at the Wisconsin Crop Innovation Center

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The Wisconsin Crop Innovation Center is committed to supporting genome research across multiple crop species with maize as a high priority. In 2018, we began receiving transformation/editing orders and initiating contracts that pushed the limits of our initial staff. In response, we continue to build our staff and to develop and utilize transformation/editing technology advancements that will allow us to meet demand in an efficient, timely, and cost-effective way. Beginning in 2019, we will implement licensed technologies, including the Japan Tobacco superbinary systems and protocols, and the Corteva Dev Gene (bbm/wus)f systems that will enhance our maize transformation/editing efficiency and capacity. We will focus on the B73 v4 inbred as the primary initial fee-for-service product, and will consider one to two additional standard lines. With these technologies, we can theoretically transform other inbreds as well, but would negotiate these on a per project basis, as it is logistically challenging to maintain a large number of options as a core service. We have also completed a license with Bayer for maize technologies to be used for public researchers only and anticipate that an additional elite maize inbred will be enabled through this process. Our molecular technologies department uses Golden Gate assembly to build binary vectors, has invented new Golden Gate tools which mediate high throughput construction of gene editing vectors, and will soon implement the GAANTRY system. We are working to utilize these technologies as quickly as possible. We appreciate your patience and support as we grow to be the type of the facility that will support high-throughput public projects. We are excited that the new licenses that we have obtained provide FTO options for small/medium-sized companies to advance their products toward commercialization through research in monocots. We appreciate any feedback and ideas from the maize research community.

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

P116 

## Exploring the use of NIRS to predict starch in whole seed pea

(submitted by Gokhan Hacisalihoglu <[gokhan.h@famu.edu](mailto:gokhan.h@famu.edu)>)

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Pea (*Pisum sativum*) is one of the most important legume crops worldwide for its rich seed composition. This study aimed to evaluate the potential usage of near infrared reflectance spectrometer (NIRS) to predict starch content in whole pea seeds. The n=288 pea seeds (96 genotypes x 3 biological reps) were analyzed using both NIRS and chemometrics methods. NIRS is a non-invasive, cost-effective and rapid technique that allows simultaneous determination of multiple seed traits such as protein, oil, weight, and starch. Single seed weights and NIR spectra were recorded at 1nm intervals with a NIR256 spectrometer. Seeds were ground and total starch was determined by enzymatic hydrolysis (alpha-amylase & AMG), GOP-aided colorimetric determination at A510. PLS regression models of calibration were developed for various seed traits. Our results revealed that NIRS shows promise, significantly reduce cost, and time for predicting seed traits in pea. The current status of this project will be presented including the further research results and analysis.

Funding acknowledgement: National Science Foundation (NSF)

P117

## Expression analysis for cuticular wax gene discovery and regulatory networks

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Cuticular waxes constitute the outermost layer of aerial epidermis of land plants and as such provide protection from environmental stresses. A handful of maize genes, the so-called glossy genes, which are responsible for the biosynthesis and accumulation of waxes, have been cloned. Following an expression analysis of RNA-Seq datasets from diverse tissues, we found that glossy genes are co-expressed, generally being active in young leaves, silks, and meiotic tassels, and maintain lower levels of activity in seeds and roots. Our pathway-level co-expression analysis identified a set of transcription factors potentially regulating co-expression of the glossy genes. These transcription factors include a cloned glossy gene, *gl3* and multiple homologs of known wax accumulation regulators. Utilizing designer transcription activator-like (TAL) effectors, we will establish a transient expression system in maize to identify downstream genes of transcription factors. In addition, RNA sequencing data was derived from mutants of eight glossy loci that result in reduced accumulation of cuticular waxes. Genes in the wax pathway and stress-responsive genes tended to be either down-regulated or up-regulated in glossy mutants, respectively. With the aid of expression data, the involvement of two new maize genes in wax accumulation was identified and later validated with the glossy phenotype observed from additional independent mutants of these two genes. The results deepen our understanding of the transcriptional regulation of the genes involved in cuticular wax production and provide a new strategy through co-expression analysis to accelerate gene discovery. We anticipate that this strategy could be extended to the analysis of other gene regulatory networks.

Funding acknowledgement: National Key Research and Development Program of China, the National Natural Science Foundation of China, the Agricultural Science and Technology Innovation Program of CAAS



P118 

## Fine-tuning different transcriptional complexes to regulate anthocyanin pigmentation genes

(submitted by Nan Jiang <[jiangn11@msu.edu](mailto:jiangn11@msu.edu)>)

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In the maize aleurone layer, the basic helix-loop-helix (bHLH) transcription factor R interacts with the R2R3-MYB regulator C1 to activate anthocyanin biosynthesis pathway. However, it is unclear how the coordinate regulation is accomplished, without significant conservation of the regulatory regions of the pathway genes. Our previous studies showed that the monomer/dimer configuration of an ACT domain<sup>1,2</sup> at the C-terminus of R affects DNA-binding of the R bHLH domain. When the ACT domain forms a dimer (ACT-ON), the bHLH is monomeric and R is tethered to DNA indirectly, through the interaction with C1<sup>3</sup>. When the dimerization of the ACT is impaired (ACT-OFF), then the bHLH domain dimerizes and R recognizes canonical G-box DNA motifs directly through the dimeric bHLH. Preliminary results indicate small molecules, including flavonoid pathway intermediates, contribute to the formation of different transcriptional complexes (ACT-ON and ACT-OFF) through interactions with the ACT domain. To investigate the *in vivo* consequences of flavonoid pathway intermediates on mRNA accumulation of pathway genes, we examined multiple pathway mutants by Nanostring and RNA-Seq approaches. Interestingly, most pathway genes are down-regulated in *bz1* mutants, with the opposite effect in *a2*. Using amplified luminescent proximity homogeneous assay (ALPHA), we have started to determine the parameters associated with protein-protein and protein-DNA interactions and the influence of small molecules. Funding for this project is provided by NSF MCB-1513807 and MCB-1822343

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Gene / Gene Models described: *C1*, *R*; GRMZM2G005066/Zm00001d044975, GRMZM5G822829/Zm00001d026147

Funding acknowledgement: National Science Foundation (NSF)

P119

## Flowering delay and *Aspergillus flavus*-resistance are correlated with increased corn earworm damage and enhanced seed fumonisin in maize

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A preference in the natural infestation of corn earworm (CEW: *Helicoverpa zea* Boddie) to specific host genotypes was observed in field trials to assess the pathogenicity of *Aspergillus flavus* strains in two maize (*Zea mays* L.) hybrids and inbreds with contrasting levels of resistance to aflatoxin (AF) accumulation. The resistant hybrid (Mp313E × Mp717) had greater than 14-fold infested ears than the susceptible hybrid (GA209 × T173). Similarly, the resistant inbred (CML322) had greater than 7-fold heavier CEW infestation than the susceptible inbred (B73). In addition AF contamination below the levels of toxicity to CEW, these two maize lines with heavy infestation showed delayed silk emergence. Since *H. zea* oviposits directly on silks, the availability of green silk for egg-laying in the late flowering genotypes may be one of the contributing factors to their preferential infestation. The level of CEW infestation had little influence on seed AF levels either in uninoculated ears or in ears inoculated manually with toxigenic *A. flavus* strains. Although no manual inoculation with *Fusarium* species was carried out, the CEW-infested ears showed a significantly greater seed fumonisin (FUM) content compared to uninfested ears from plots that were mock-inoculated or inoculated with *A. flavus*. In spite of its superior resistance FUM accumulation demonstrated previously, Mp313E × Mp717 had greater levels of FUM than GA209 × T173 correlating with the level of CEW infestation. In summary, mycotoxin contamination of crops is determined not only by the level of a host resistance to the cognate fungus, but also phenological traits that compromise its resistance.

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

P120 

## Functional annotation of maize auxin repressor proteins

(submitted by Yuli Buckley <[buckley@whitman.edu](mailto:buckley@whitman.edu)>)

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The hormone auxin plays a key role in growth and developmental pathways in all plants. In maize, auxin is directly involved in tassel and ear development. Nuclear auxin signaling is comprised of a seemingly simple pathway consisting of a receptor, repressor, co-repressor, and transcription factor. However, each pathway component has many gene family members. In maize, 16 different putative auxin repressors are expressed in developing ears and tassels. In order to functionally annotate each of these maize auxin repressors (ZmIAAs), we have utilized a yeast-based recapitulation of auxin signaling. To test the repression ability of each repressor, the ZmIAAs were co-expressed in yeast along with a fragment of the maize transcriptional corepressor RAMOSA1 ENHANCER LOCUS2 (REL2), a member of the highly-conserved TPL family. Each yeast strain additionally contained the other auxin pathway components (derived from the Arabidopsis auxin signaling pathway) and an auxin-responsive promoter driving expression of a fluorescent reporter. ZmIAAs fused to a short fragment of REL2 were able to repress auxin-dependent gene expression with varying levels of efficiency that correlated with the expression level of the ZmIAA. Upon treatment with auxin, these repressor fusions were degraded and differential fold induction of gene expression was observed. The level of induction correlated to the rate of auxin-induced ZmIAA degradation. These patterns of differential repression and relief of repression are comparable to those observed for Arabidopsis auxin repressors. A further understanding of this pathway can help enhance our knowledge of how to engineer growth and development of maize plants.

Gene / Gene Models described: ; REL2, BIF1, ZmIAA8, ZmIAA2, ZmIAA12, ZmIAA16

Funding acknowledgement: National Science Foundation (NSF), MJ Murdock Charitable Trust, Whitman College

P121 

## Functional characterization of maize auxin signaling modules in yeast

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The hormone auxin regulates myriad processes during the life of a plant - from root and shoot development to environmental responses. Understanding how auxin regulates such diverse processes necessitates characterization of the specific signaling modules (receptors, repressors, transcription factors) that enable plant cells to detect and respond to auxin. Recapitulation of minimal auxin signaling modules from *Arabidopsis* has been successfully accomplished in yeast. These studies have revealed that auxin repressors (Aux/IAAs) exhibit a range of auxin-induced degradation rates which can be tuned depending on identity of the co-expressed auxin receptor, and that this natural variation in Aux/IAA degradation is central to controlling auxin transcriptional response dynamics. Subsequent studies confirmed that Aux/IAAs show similar degradation differences *in planta* and that Aux/IAA degradation dynamics are highly correlated with the rate of developmental events. We are now using this yeast system to functionally annotate auxin signaling modules crucial during maize reproductive organogenesis. We have identified the subset of maize Aux/IAAs expressed in developing inflorescences and have utilized the yeast system to reveal that these repressors: (1) degrade in response to auxin, (2) exhibit a wide-range of auxin-induced degradation rates, and (3) can repress transcription with the assistance of the maize co-repressor protein REL2. Much of this work was carried out by nearly 30 undergraduate students at Whitman college, working as summer interns or in course-based undergraduate research experiences. Current research efforts are centered on assembling fully-maize auxin signaling modules (using maize auxin receptors and transcription factors) and interrogating the molecular determinants of maize Aux/IAA degradation dynamics. This work is providing new insights to inform our understanding of how auxin action is specified by context-specific deployment of auxin signaling components during plant development.

Funding acknowledgement: National Science Foundation (NSF), MJ Murdock Charitable Trust, Whitman College

P122 

## Genetic and metabolomic analysis of maize lesion mimic mutants and hypersensitive response

(submitted by Ryan Benke <[rbenke@purdue.edu](mailto:rbenke@purdue.edu)>)

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The hypersensitive response (HR) to incompatible pathogens results in cell death at points of attempted pathogen infection. This immune response is found in all multicellular plants. Despite this prevalence and studies of the transcriptional, metabolomic, and proteomic changes associated with HR, analysis of the genetic regulation of this response has just begun. Many studies of HR occur in the presence of pathogens, which confounds analyses of the plant response as effector proteins released by the pathogen can have multiple effects on the host organism. Some mutants spontaneously form lesions as a result of constitutively-induced HR in the absence of pathogen infection. Found in many plant species, these lesion-mimic mutants are an ideal model system for the study of HR as there is no need to control for the pathogen and all changes can be attributed to the genetics and metabolism of the plant. I am studying multiple lesion mimic mutants of *Zea mays*. The goal of this project is to characterize the remodeling of the maize transcriptome and metabolism as a result of HR and to molecularly identify genes controlling HR. Here, we present preliminary results for multiple the lesion mimic mutants with contrasting etiologies. Untargeted metabolomic profiling identified hyperaccumulated mass features in mutants with defective NLR-signaling consistent with salicylic acid and dihydroxybenzoic acids while RNA-sequencing suggests these mutants have increased flux through the phenylpropanoid pathway compared to wild type. We are attempting to discriminate between the cause of lesion formation in mutants (e.g. HR induction by NLR signaling vs phototoxic metabolite accumulation) via the inexpensive and reproducible analysis of metabolite mass features via LC-MS. Our preliminary work contrasts mutants of known molecular mechanism and determines candidate loci for lesion mutants of unknown etiology to aid in the molecular identification of alleles responsible for lesion mutant phenotypes.

Funding acknowledgement: National Science Foundation (NSF)

P123 

## Genetic architecture of Glutamine variation in Arabidopsis seeds

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Glutamine is an amino acid of particular interest because of its role as a primary metabolite in nitrogen assimilation. Assimilated nitrogen serves as substrate for additional proteinous amino acids that fuel the plant during development. Toward the end of development, free and bound amino acids are deposited into the seed. Understanding the control of nitrogen assimilation and transport to the seed is of particular interest for both nitrogen use efficiency efforts, as well as, reprogramming of seed composition for nutritional purposes. Despite attempts to rebalance seed amino acid profiles, however, the mechanism behind such reprogramming is largely unknown. In the present study, we leverage the genetic variation of the Arabidopsis 360 diversity panel in a GWAS aimed at shedding light on underlying genetic architecture of amino acid composition in the seed. We utilized derived amino acid traits calculated from ratios of absolute amino acid levels from Glutamine (numerator in derived traits), in addition to quantified amino acids levels from the Glutamate Family (denominator in derived traits) to isolate putative control mechanisms that may aid in amino acid regulation to sink tissues. We hope these findings can be leveraged in future studies to more completely understand the complex process of amino acid regulation in the seed.

Funding acknowledgement: National Science Foundation (NSF)

P124 

## Genetic architecture of source-sink regulated senescence

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Source sink communication is a key determinant of senescence, but the molecular mechanisms underlying such regulation are poorly understood. We systematically characterized the natural diversity for source-sink regulated senescence (SSRS) captured in US dent maize and a biparental population. We also conducted time-course transcriptome and metabolite analysis of SSRS sensitive and a resistant line. The chromosomal introgressions associated with SSRS were mapped to chromosomes 1 and 5 and further narrowed down with backcrossing. The candidate genes related to the GWAS SNPs and QTL span those involved in DNA regulatory functions, sugar transport and signaling, sink activity, and proteolytic processes. Majority of the candidates are novel genes and previously have not been reported for their direct role in SSRS. The transcriptome analysis of SSRS sensitive and resistant line identified differentially expressed genes including those identified by genomic analyses. The metabolite analysis provides crucial insights into the role of hormones as signaling molecules in SSRS. Finally, our study demonstrated mechanistic similarities between SSRS and natural senescence. Therefore, the genetic architecture of SSRS provides a foundation for understanding and manipulation of natural senescence for increasing carbon yield and making stress resilient crops.

Funding acknowledgement: United States Department of Agriculture (USDA)

P125 

## Genetic basis of seed free and protein bound amino acid pools and their metabolic relationship in maize

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Seeds are a major source of protein in human and livestock diets. However, the seeds of major staple crops such as maize, soybeans and rice are deficient in several essential amino acids (EAA). Failure to consume sufficient levels of EAA per day leads to severe malnutrition, even if one's calories requirements are met. So far, limited successes has been achieved in improving the seeds amino acids in either classical or transgenic approaches since these traits are tightly regulated. In fact seeds actively rebalance their composition even when severe perturbation to the seed protein content and composition is introduced by transgenic measured. However, the genetic identity of this mechanism is not clear. Nevertheless seed amino acid composition display extensive natural variation which can be exploited to uncover how nature genetically regulates these essential traits. To this end we characterize the natural variation of these traits across 282 genetically diverse lines of maize using advance high-throughput analytical methods and associate it with the natural genetic variation using genome wide association study (GWAS). More specifically, we focus on the characterization of two functional pools of amino acid and their potential interplay: the free amino acid pool, which comprises ~5% of the total amino acid in seeds and the protein bound amino acid pool, which comprises ~95% of the total amino acid in seeds. We will be presenting the candidate genes regulating the free and bound amino acids and their metabolic relationship in dry seeds. Uncovering the genetic basis of both amino acid functional pools as well as their interplay may open new avenues to seeds biofortification.

Funding acknowledgement: National Science Foundation (NSF)

P126 

## Genetic control of carbon isotope ratios in maize kernels

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Carbon isotope discrimination is widely used to distinguish C3 and C4 photosynthesis. The differences in bending and vibration of CO<sub>2</sub> containing <sup>12</sup>C, rather than the heavier <sup>13</sup>C isotope, during the fixation of carbon by RUBISCO results in a higher ratio of <sup>12</sup>C in the 3-phosphoglycerate pools and tissues of C3 plants than is found in the atmosphere. The ability to discriminate between the isotopomers of CO<sub>2</sub> is reduced during water stress in C3 plants, as stomates remain closed until all carbon is fixed, rather than constantly replenishing unfixed atmospheric CO<sub>2</sub>. Some controversy exists as to whether drought response in C4 plants can also be assessed by carbon isotope discrimination. Classic plant physiology demonstrated that variation in discrimination by C4 plants is primarily driven by leakage from the bundle sheath, and will not be impacted by water relations. Other work has suggested an effect of drought on discrimination. In order to test these hypotheses, and to identify genetic loci associated with carbon isotope discrimination, a panel of 299 diverse maize inbred lines was grown under contrasting irrigation regimes in Chile. Kernels were sampled from each plant at maturity, in an effort to provide an integrative measure of photosynthesis over the period of grain filling. Carbon isotope contents were determined. Carbon discrimination values were heritable and were correlated between treatments. This is consistent with the leakage model and not with the use of carbon isotopes to identify drought tolerance in maize. In addition, despite the high heritability, genome-wide association analysis did not identify any significant associations after multiple testing correction. From these results, we draw two conclusions. First, carbon isotope discrimination in maize kernels is predominantly under genetic, rather than environmental, control. Second, carbon isotope discrimination is likely affected by a large number of loci, each of small proportionate effects, that integrate photosynthetic carbon assimilation over all plant tissues.

Funding acknowledgement: National Science Foundation (NSF)

P127

## Genetic control of guard cell Movement via the CO<sub>2</sub> stomatal signaling pathway in *Zea mays*

(submitted by Robert Twohey III <[twohey2@illinois.edu](mailto:twohey2@illinois.edu)>)

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Plants uptake CO<sub>2</sub> through their stomatal pores for photosynthesis. Simultaneously, there is a corresponding efflux of water via transpiration. Stomata control this CO<sub>2</sub> and transpiration equilibrium by altering their aperture size via guard cells. Stomata respond to various environmental factors such as atmospheric CO<sub>2</sub> levels, humidity, light, and temperature. The CO<sub>2</sub> stomatal signaling pathway was previously elucidated in Arabidopsis through mutant analysis of multiple genes. The monocot pathway is thought to be similar, but not identical to the pathway found in dicot species. Here we present the characterization of genes involved in the CO<sub>2</sub> signaling pathway in *Zea mays*. *Slac1* (GRMZM2G106921) an S-type anion channel, and two orthologs of the Arabidopsis gene OST1, named Open stomata (*Opst1*, GRMZM2G138861 and *Opst2*, GRMZM2G171435), which are SnRK2 protein kinases. Mutant alleles were identified in *Z. mays* from the UniformMu collection and were sequence validated. Amplification of mutant cDNA showed that the *slac1-1* allele results in a truncated, non-functioning protein and the *slac1-2* allele results in a complete gene knockout. Leaf level gas exchange measurements were used to look at the physiological response of the mutants to environmental stimuli. *slac1-1* and *slac1-2* mutants show a significant insensitivity to changes in CO<sub>2</sub>, resulting in a more open stomata phenotype, even under high CO<sub>2</sub> levels. The *opst1* and *opst2* single mutants were sensitive to atmospheric CO<sub>2</sub>, resulting in stomatal response phenotypes identical to wild-type plants. The characterization of *opst1/opst2* double mutants will give insight into the degree these paralogs have redundant functions. Dissection of the CO<sub>2</sub> stomatal signaling pathway will provide new insights into the optimization of stomatal regulation. Given current climate trends, the production of more efficient crops, by reducing transpiration, is increasing in importance for sustainable agriculture production.

Gene / Gene Models described: ; GRMZM2G106921, GRMZM2G138861, GRMZM2G171435

Funding acknowledgement: United States Department of Agriculture (USDA)



P128

### Genetic dissection of host resistance to Goss's Wilt in maize

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Goss's bacterial wilt of maize, caused by the Gram-positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis*, has spread in recent years throughout the Great Plains. The genetic basis of plant resistance to Goss's wilt or to other diseases caused by Gram-positive bacteria, in general, is poorly understood. Here, the cohorts of resistant (R) and susceptible (S) lines were selected through quantification of disease symptoms on more than 600 maize lines. Whole genome sequencing of these individual R and S lines as well as RNA-seq of separate pools of R and S lines were then performed. Whole genome sequencing was used for genome-wide association analysis and pooled RNA-Seq was used to identify genes exhibiting differential allelic responses to the bacterial treatment. Both results were combined along with quantitative trait loci mapped using bi-parental segregation populations to identify candidate disease defense loci, two of which were verified via phenotypic comparison between near-isogenic lines. In addition, R and S lines were crossed to produce mapping populations. Genetic mapping was conducted using F2 populations showed 3:1 R:S segregation. Our analyses provide genetic information to further examine this underexplored phyto-bacterial system, as well as resistant resources for breeding Goss's wilt resistant maize varieties.

Funding acknowledgement: United States Department of Agriculture (USDA)

P129 

### Genetic diversity and population structure of farmers' maize varieties (*Zea mays* L.) from selected states in Nigeria using SSR markers and their relationship with standard hybrids

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Maize (*Zea mays* L.) is an economically important tropical crop cultivated by farmers in Nigeria. In this study, genetic diversity and population structure were assessed in a set of 19 maize varieties, including most currently grown maize farmers' varieties collected from towns/villages in Edo, Ekiti and Kwara States in Nigeria and recently developed International Institute of Tropical Agriculture (IITA) maize hybrids based on 20 SSR flanking markers of previously mapped quantitative trait loci linked with the oil and protein or oil/protein contents. All SSR loci detected in all genotypes were polymorphic. Based on the SSR genotyping data, a total of 183 alleles (ranging from 3 to 18; mean 9.15) with the mean polymorphism information content value of 0.75 (ranging from 0.43 to 0.90) were observed. A moderately high number of unique alleles was present in 3 farmers' varieties and a hybrid. The 19 maize varieties were separated into two main groups: 5 varieties clustered in group I; 13 maize varieties clustered in group II but subdivided into two subgroups; three hybrids (LW17, LW18, and LW19), and 4 farmers' varieties from Kwara State clustered in subgroup 2 in the dendrogram. The one landrace from Ekiti State was distinct from the other farmers' varieties and the newly developed hybrid lines. The result from the factorial analysis was consistent with the dendrogram and the States of the collection of farmers' varieties and the newly developed hybrid was considered substantial. The result of the STRUCTURE analysis classified the 19 varieties as an admixture, thus produced one population which was not in accordance with the dendrogram and factorial analyses. The study using SSR analysis has revealed the genetic variation among the farmers' varieties' and their genetic relationship with the IITA maize hybrids. The information obtained from the set may form the basis for maize breeding and conservation programs in the future.

Key Words: Genetic diversity and structure, *Zea mays* L., SSR markers, IITA hybrids, and farmers' varieties

Funding acknowledgement: Tertiary Education Trust fund (TETFUND) Grant, Nigeria (CRC/TETFUND/NO2017/07)221-6-00).



P130 

## Genetic mapping and characterization of the nitrate transporter (NRT1.1) gene family in maize

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An important component to increasing crop productivity is improving Nitrogen Utilization Efficiency (NUE). In maize this trait is measured as the ratio of grain yield to accumulated plant N. Enhancing NUE offers substantial economic and environmental benefits, but little is known about the genetic mechanisms that govern variation for NUE within maize populations. Our group has conducted high density genetic mapping for NUE in a hybrid population developed from the intermated B73 X Mo17 recombinant inbred lines (IBMRILs), test crossed to the Illinois High Protein 1 (IHP1) inbred line, which has a superior capacity for N uptake but low NUE. Of the 9 genomic regions found to be associated with NUE in this study, the largest effect QTL is localized to a 2 Mb region on chromosome 1. This QTL contains 23 annotated genes, including the high affinity nitrate transporter NRT1.1 B. Both NRT1.1B and one of its homologs, NRT1.1A, have recently been shown to have a significant effect on NUE traits within rice. However, little research has been done on how these two genes, as well as other members of the NRT1.1 gene family, are functioning within maize. Currently, no mutant allele of NRT1.1 B is available through public repositories; however, an Ac/Ds transposon insertion is available in maize NRT1.1A. Maize lines containing homozygous transposon insertions in NRT1.1A were phenotyped for various traits associated with NUE during the 2018 field season. Differences in stover nitrogen content, plant vigor and various root traits were observed between mutant and wild type plants. In order to further elucidate the function of these nitrate transporters in maize, the Moose lab is employing Crispr-Cas9 to target additional members of the NRT1.1 gene family.

Funding acknowledgement: United States Department of Agriculture (USDA)

P131 

## Genetics underlying the multiple aleurone layer trait in maize

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The aleurone layer is the outermost layer of the endosperm and plays an important role in germination as it digests the components necessary for a developing seedling. The aleurone layer is also important in terms of nutrition as it is a nutritive fraction of the kernel. Typically, aleurone is a single cell layer around the peripheral of the maize endosperm. However, certain landraces of maize contain 2-6 aleurone cell layers. The genetics underlying this trait are complex and currently unknown. The aim of this study was to uncover the complexity of this trait and find loci associated with multiple aleurone layer formation. A single backcross population was made with recurrent parent Mo17 and landrace San Martin 105 with four aleurone layers. Results of the population showed that 73.8% BC<sub>1</sub>F<sub>1</sub> ears were capable of producing at least a few doubled aleurone cell layers, indicating at least two genes are involved with the trait. Of the BC<sub>1</sub>F<sub>2</sub> kernels analyzed, only 4.8% were capable of producing three cell layers. With QTL analysis, genetic markers were discovered that were linked to the trait. To test the robustness of these markers, a single backcross population was made between recurrent parent genetic stock 707G and multiple aleurone layer accession San Martin 119. The BC<sub>1</sub>F<sub>2:3</sub> families developed segregated for the *intensifier1* gene conferring intense blue aleurone pigmentation and for multiple aleurone layer formation. The genetic markers for multiple aleurone layer formation were also tested for their association with anthocyanin content. Results show that increasing aleurone layers can increase kernel anthocyanin content and the nutritional value of maize.

P132


## Genome-wide association mapping for anthocyanin composition in purple corn (submitted by Laura Chatham <[lachatham@gmail.com](mailto:lachatham@gmail.com)>)

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Pericarp-pigmented anthocyanin-rich purple corn has been identified as an economical yet natural alternative to artificial colorants. Anthocyanins are water-soluble and extracts range in color from orange to pink to red, making them a viable replacement for some artificial colorants if appropriate hues can be matched. The anthocyanidin (cyanidin, pelargonidin, or peonidin) from which each anthocyanin is derived plays a significant role in the color of maize kernel extracts. Most of the diversity and research in anthocyanin-containing maize is associated with aleurone-pigmented lines; however, pigmented-pericarp varieties allow for more efficient pigment extraction and can produce concentrations often an order of magnitude greater than aleurone lines. A pericarp-pigmented landrace with variability in anthocyanin composition was repeatedly self-pollinated to exploit natural variability, and association mapping was performed on the resulting population to identify loci associated with anthocyanin biosynthesis. A major QTL for all three anthocyanidin types was found near *red aleurone1 (pr1)*, suggesting this gene functions similarly in both aleurone and pericarp varieties. Additionally, a significant QTL associated with peonidin-derived anthocyanins near a candidate S-adenosylmethionine-dependent methyltransferase was identified, warranting further investigation. The loci identified in this population may be useful in the creation of molecular markers that aid in breeding for altered flavonoid profiles in maize.

Gene / Gene Models described: *pr1*; GRMZM2G025832

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## GIF1 controls the ear inflorescence architecture and floral development by regulating RAMOSA and CLAVATA-WUSCHEL pathway in maize

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Inflorescence architecture and normal floral development in the flowering plant species are closely determined by those genes controlling meristem identity, determinacy and maintenance. The female inflorescence (the ear) meristem in maize initiates a type of short branch meristem: spikelet pair meristem (SPM) which then differentiates into determinate spikelet meristems, resulting in lack of long branches on normal ears. However, loss of function mutants in maize *growth-regulating factor-interacting factor1*, *gif1*, generated highly branched ears, and those extra branches could repeatedly produce branches or differentiate determinate florets with unfused carpel and expanded nucellus. By creating functionally complementary transgenic lines using GIF1::GFP fusion construct, we identified GIF1-interacting proteins using Co-Immunoprecipitation, and GIF1-targeting genes by integrating Chromatin Immunoprecipitation sequencing and transcriptome data. We found that GIF1 regulates the ear inflorescence branching through targeting RAMOSA pathway, controls activity of ear inflorescence meristem by *ZmCLE4a*-mediated CLV-WUS feedback loop, determines pistillate floret development by interacting with *nana plant2(na2)* to influence hormone homeostasis. We propose that GIF1 is requisite for identity of SPM, activity maintenance of inflorescence meristem and carpel margin meristem on the ears.

Gene / Gene Models described: *gif1*, *ZmCLE4a*, *nana plant2*; Zm00001d033905, Zm00001d034507, Zm00001d014887

Funding acknowledgement: National Key Research and Development Program of China, National Natural Science Foundation of China

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## **Global modulation of gene expression by genomic imbalance caused by a dosage series of chromosome arm 5S**

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It has been long known that genome imbalance caused by changing the dosage of individual chromosomes (aneuploidy) has a more detrimental effect phenotypically than by varying the dosage of complete sets of chromosomes (polyploidy). Previous work from the laboratory examined individual genes for effects of changes in chromosomal dosage. The predominant effect was an inverse modulation in trans across the genome but positive modulations were also observed. Here we extend such studies to global examination of such effects. The genome has been surveyed using a collection of B-A translocations via RNA-seq studies of maize mature leaf tissue. One of the most dramatic effects results from a dosage series of the short arm of chromosome 5 (5S). Within diploids, monosomics, trisomics, and tetrasomics were compared to the normal diploid. In haploids, disomic 5S was compared to haploid controls. The results indicate that significant changes in gene expression occur both on the varied chromosome (cis) and the remainder of the genome (trans). In general, cis genes range from dosage compensation to a dosage effect, whereas trans genes largely show an inverse correlation in that expression decreases with increased doses of chromosome 5S. Comparisons across ploidies show much less modulation. Regulatory network analysis of RNA-seq data demonstrates changes in global gene expression could be caused by gene regulatory cascades triggered by the varied copy number of 5S. In addition, expression analyses were performed on transposable elements, microRNAs and small interfering RNAs to examine their roles in genome imbalance. This study will provide insight into the underlying molecular mechanisms involved in maintaining genomic balance and how regulatory dosage effects operate transcriptionally and post-transcriptionally. Funding from NSF IOS-1545780.

Funding acknowledgement: National Science Foundation (NSF)

P135

## **GRMZM2G145104, a RING E3 domain containing ubiquitin ligase partially suppresses Rp1D21 induced cell death in maize**

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In order to defend against diverse microbial pathogens, plants rely on an innate immunity strategy which comprises recognition of pathogen elicitors by membrane bound pattern recognition receptors and intracellular nucleotide binding leucine-rich repeat receptors (NLRs) that recognize specific proteins produced by the pathogen to facilitate pathogenesis. This recognition initiates a defense response often including the so-called hypersensitive response (HR), a rapid localized cell death at the point of pathogen penetration. In maize, the intragenic recombination of two NLR's, Rp1D and Rp1dp2 produced an auto-active NLR, Rp1D21 which confers a spontaneous HR phenotype. A genome wide association study identified several SNP loci associated with variation in the Rp1D21-induced HR response. One was located within a gene GRMZM2G145104, encoding a predicted RING E3 ubiquitin ligase. Transient co-expression studies in *Nicotiana benthamiana* showed suppression of Rp1D21-induced HR by GRMZM2G145104 coupled with a decrease in the levels of Rp1D21 protein suggesting that Rp1D21 may be a plausible target for GRMZM2G145104-mediated ubiquitination. Similarly, GRMZM2G145104 co-expression reduced the levels of another auto-active NLR, RPM1D505V. Interestingly, additional co-expression studies indicated that co-expression with GRMZM2G145104 did not decrease protein levels of Rp1D and Rp1dp2. These results suggest that GRMZM2G145104 may specifically target auto-active but not 'wild-type' NLRs for degradation.

In related work using chimeric mutants we demonstrated that while the HR induced by Rp1D21 is cell autonomous, the defense response signal that induced expression of pathogenesis-related genes is not.

Gene / Gene Models described: *Rp1D21*; GRMZM2G145104

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

P136

## High frequent DNA rearrangement at the 27-kD $\gamma$ -zein locus creates a superior *o2* modifier for quality protein maize breeding

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Copy number variation is a major source of genetic variation between individual maize inbred lines and often contributes to phenotypic variation in agronomic traits. The duplication at the 27-kD  $\gamma$ -zein locus is a major *o2* modifier (*qy27*) for endosperm modification in Quality Protein Maize (QPM). This duplication is unstable and frequently produces single copies. Due to the lack of effective phenotypic or molecular markers, the frequency of germinal DNA rearrangement at this locus was incapable of being determined. 0707-1 is a primer pair that flanks a deletion in the duplicated region and K0326Y-Del is a mutant QPM line that eliminates the entire *qy27* locus, thereby no PCR band being amplified from this allele by 0707-1. When different lines were crossed to *K0326Y-Del*, the occurrence of DNA rearrangement could be determined by the PCR banding only produced from the examined lines. This genetic designation enabled us to measure DNA rearrangement frequencies occurring in pollen and embryo sac of different genetic backgrounds. The frequency with which the *qy27* duplication rearranges to single copies from one generation to another is on the order of  $10^{-3}$  and varies dramatically among different lines, with the highest in A188 and lowest in Mo17. It occurs significantly higher from male than from female in all lines detected. Since the frequency in W22 is close to the order of  $10^{-2}$ , the triplication is expected to be identified in a small number of different UniformMu stocks (W22 background). Indeed, five out of 104 stocks were determined to bear a triplication. The greatly enhanced 27-kD  $\gamma$ -zein by the triplication allele is sufficient to confer a complete endosperm modification. Our results directly determined the frequency of DNA rearrangement resulting in copy number variation at the 27-kD  $\gamma$ -zein locus and created a single superior *o2* modifier allele for future QPM breeding

Keywords: Quality Protein Maize (QPM); *o2* modifier; Copy number variation; DNA rearrangement

Gene / Gene Models described: *27-kDa  $\gamma$ -zein*; GRMZM2G138727

Funding acknowledgement: The National Natural Science Foundation of China (31771799 to H.L., 31830063 to Y. W)

P137 

## Hyperspectral estimation of biological nitrogen fixation in dry beans

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Dry bean (*Phaseolus vulgaris* L.) is an important herbaceous annual crop grown throughout North America. It is considered a poor nitrogen fixator in comparison with other leguminous crops, requiring supplemental nitrogen fertilizer for profitable dry bean production. Because breeding and selection have been conducted under high nitrogen fertility, many modern varieties show significantly reduced ability to fix nitrogen. However, genetic variation has been observed among genotypes of dry beans for nitrogen fixation. Thus, it is possible to evaluate the performance of genotypes for the optimization of nitrogen fertilizer management to improve crop production and reduce excess nitrogen use. Though it is possible to measure nitrogen directly, a high-throughput phenotyping method provides a way to cheaply and rapidly screen populations of lines. In this study, hyperspectral imaging technology is utilized to scan dry bean plants in order to estimate nitrogen status. The objectives of this study are (1) to analyze the accuracy of different wavelengths and models for estimation of nitrogen status, and (2) model dry bean biomass using nitrogen status and spectral features. A dry bean population with a wide range of genetic variation was grown in the greenhouse at Michigan State University under +/- nitrogen treatments. Flowering time and partitioned biomass were recorded. At the time of biomass collection, hyperspectral images were taken of all above-ground plant tissues. Partial least squares regression models and spectral indices were explored to predict the genotypic response to nitrogen treatment. Future work will extend these predictive models to spectral imaging from Unmanned Aerial Systems (UAS; drones). These findings will not only allow breeders to identify genotypes having high potential for nitrogen fixation, but also enable farmers to have precision management of nitrogen applications during the growing season.

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## Identification of a novel locus controlling seed tocopherol content by comparative GWAS in *Arabidopsis thaliana* and *Zea mays*

(submitted by Elise Albert <[alberte2@msu.edu](mailto:alberte2@msu.edu)>)

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Tocopherols (vitamin E) are lipid-soluble antioxidants synthesized only by photosynthetic organisms and their dietary intake, primarily from seed oils, is essential for optimal human health. The core vitamin E (VTE1 through VTE6) biosynthetic enzymes have been cloned in model organisms (*Arabidopsis thaliana* and cyanobacteria) and orthologs are readily identified in genomes of crop species like maize. Surprisingly, genome-wide association in the NAM maize panel showed that the six VTE genes are responsible for only a small part of the genetic variation in maize grain and instead, novel loci are major contributors. Of the 23 novel tocopherol loci in NAM only six were resolved to the gene level, including two major effect QTL that mapped to protochlorophyllide reductases. In a parallel GWA study of natural variation for seed tocopherols in 814 diverse *Arabidopsis* ecotypes we identified a total of 22 QTL and, similar to maize NAM, only three of these intervals contained a VTE pathway gene. The largest effect QTL mapped to a single gene on Chr 5 (termed AtVTE7) that had a corresponding ortholog in one of the unresolved NAM tocopherol intervals. YFP fusions of both AtVTE7 and ZmVTE7 localize to chloroplasts. Relative to wild type, knockout alleles of AtVTE7 decreased tocopherols by 60% and chlorophylls by 50% in dry and developing seeds, respectively. Finally, over-expression of ZmVTE7 in the AtVTE7 knockout background restored the wild type tocopherol phenotype. These preliminary results suggest that AtVTE7 and ZmVTE7 are functional orthologs that link chlorophyll metabolism and tocopherol synthesis/accumulation in monocot and dicot seeds. Experiments are ongoing to elucidate the underlying mechanism.

Funding acknowledgement: National Science Foundation (NSF)

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## Identification, classification, and analysis of AT-Hook family in maize

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AT-hook motif nuclear localized (AHL) genes, a conserved group of protein-coding genes in land plants, have diverse biological functions that are poorly understood. To better understand their diversity and biological function, we identified, analyzed, and classified AHL genes in the latest version (B73\_RefGen\_V4) of the maize genome. A total of 37 AHL proteins were identified and classified into 3 types based on the predicted protein sequence and the presence of critical functional domains. Phylogenetic analysis of AHL genes in four plant species (maize, rice, sorghum, and Arabidopsis) provides important clues about the evolution of the AHL gene family in land plants. Gene expression profiles from our comprehensive gene atlas offered valuable information about spatial and temporal expression patterns and the various biological roles of the AHL genes. To identify the interacting partners of AHL, we constructed gene co-expression networks for the vital organ groups and specific physiological processes and identified AHL genes specific to these networks. Several AHL genes were specific to the embryo and endosperm-specific networks that indicates a crucial role of these genes in development and biological activities specific to reproduction and sink activity. Our study provides useful insights into the role of AHL genes in plant development and provides a platform for future functional analysis of these genes in maize and related grasses.

Funding acknowledgement: United States Department of Agriculture (USDA)

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## Identify the gene regulatory network responsible of phenolic biosynthesis in maize.

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Control of gene expression is an essential biological processes and has a fundamental impact on crop productivity, climate adaptation and pathogen resistance. The vast genetic and genomic resources available makes maize an excellent model system to understand the hierarchy of gene regulatory networks (GRNs). Thus, the overall goal of this project is to establish the architecture of gene expression in maize and identify the mechanisms by which transcription factors (TFs) regulate phenolic biosynthesis in maize. Previous work from our group has identified a set of 45 TFs that interact with promoters of multiple phenolic biosynthesis genes. In order to identify direct targets of these TFs in vivo, outlined a project to generate antibodies that specifically recognize the TFs in plant tissues. For this purpose, we have cloned, expressed and purified TFs recombinant proteins in *E. coli*. These antibodies will be used to dissect the TFs-DNA interactions by ChIP. These novel reagents will be used to determine the TFs expression and TFs-target regulation in different plant tissues to understand how biotic and abiotic stress conditions regulate phenolic biosynthesis. Ultimately, these studies will provide a better insight of gene expression in maize

Funding acknowledgement: National Science Foundation (NSF)



P141

## Insights into the regulation of starch biosynthesis in sweet corn endosperms.


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Sweet corn (*Zea mays* var. *saccharata*) has high sugar content due to mutations in the biosynthetic pathway that convert sucrose to starch in the endosperm. The pathway is controlled by several genes, but *sugary1* (*su1*) and *shrunk2* (*sh2*) are the main genes explored in commercial sweet corn. The *su1* allele is a leaky mutation in the gene coding for an isoamylase, a starch branching enzyme. The *sh2* mutation present in commercial hybrids produces a null mutation of the endosperm ADP-Glucose-Pyrophosphorylase (AGPase). The effect of these mutations on the regulation of this pathway is not fully characterized. Moreover, *sh2* plants still have approximately 25% of wildtype endosperm starch content. The objective of this study is to further elucidate the regulation of starch biosynthesis and understand the source of this residual starch production in the endosperm of sweet corn. An RNAseq experiment was performed on greenhouse-grown near-isogenic lines (NILs) (wt, *sh2*, and *su1*). Kernels were harvested 14 days after pollination and dissected in order to specifically study endosperm expression. Differential expression and coexpression network analyses were conducted and indicate complex changes in expression in starch biosynthesis genes as well as genes not previously included in the pathway. Trehalase and starch phosphorylase are members of the same coexpression network and appear to be upregulated by the buildup of D-glucose-1P. The expression patterns of these enzymes suggest a rerouting of the substrate that could explain residual starch found in *sh2* mutants. In order to confirm these findings, enzyme activity assays were performed in the same NILs. Understanding the role of gene expression in this residual starch production will help unravel the regulation and steps in starch biosynthesis, as well as provide potential targets for mutation to create sweeter sweet corn varieties.

Funding acknowledgement: United States Department of Agriculture (USDA)

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## Investigating transcriptional regulation of abiotic stress response in sorghum

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When compared to maize, its close relative sorghum is a relatively modest grain crop in the United States. In parts of the developing world, however, sorghum is and has long been a major grain, with total production exceeding 60 million metric tons in the 2016/2017 season. Within the US, the need to diversify our energy portfolio has sparked interest in stress tolerant biofuel crops as a source of renewable energy. Its ability to thrive with limited water and nutrient inputs, extensive genetic diversity and multiple end uses, including sugar and cellulosic biomass in addition to grain, make sorghum a prime candidate for enhanced crop improvement. Currently, there is little known about the molecular mechanisms that underlie sorghum's exceptional stress-tolerance. To this end, we performed a controlled environment experiment where we subject sorghum plants (reference grain line BTx623) to drought and low nitrogen stress treatments. We interrogated genome-wide chromatin accessibility and transcriptional changes by performing ATAC-seq and RNA-seq, respectively, in the same shoot and root tissues from control, drought- and nitrogen-stressed plants. Through integrated data analyses we defined numerous regions of the genome that were differentially accessible in response to stress, many of which were proximal to differentially expressed genes, including those previously implicated in stress response. We are continuing to mine these data through integration of available GWAS and variation data from sorghum to further prioritize regulatory regions that may confer enhanced water and/or nitrogen use efficiency. Taken together, these data provide valuable insight into sorghum's resilience to abiotic stress, which may be leveraged for crop improvement in sorghum or in other closely related cereals such as maize.

Funding acknowledgement: Department of Energy (DOE)



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## Investigation of carbonic anhydrase to improve C4 photosynthetic efficiency

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One objective of this project is to optimize the expression of carbonic anhydrase (CA) to increase the residence time of carbon dioxide in the leaf to improve C4 photosynthesis and drought resilience. Phylogenetic and gene expression analysis of CA across the grasses demonstrates that there are several  $\alpha$ ,  $\beta$ ,  $\gamma$  - CA genes present in all grass genomes that exhibit a conserved expression in leaf tissue regardless of photosynthetic type. We are working to understand the role of alternative splicing of the most abundant leaf expressed  $\beta$ -CA, with the working hypothesis that two transcript isoforms facilitate CA being localized to different sub-cellular locations. On-going experiments include investigation of CA protein targeting and biochemical analysis of the alternatively spliced protein products. Results suggest that translation of the most abundant C4 CA transcript does not begin in exon 1 as previously annotated. These results provide insights for designing constructs to optimize CA expression in photosynthetic grass leaves. Constructs that are currently being made include a  $\beta$ -CA overexpression construct and a transcriptional activator, dCas9-TV, for all  $\beta$ -CA isoform promoters with 1-2 sgRNAs per isoform. Further research on CA promoter elements that drive elevated and cell-specific gene expression, will help refine strategies for editing CA promoters to improve photosynthesis in Sorghum.

Funding acknowledgement: Department of Energy (DOE)

P144 

## Investigation of flavonoid compound accumulation in mature seeds across a set of diverse maize lines

(submitted by Mine Gezin <[gezginmi@msu.edu](mailto:gezginmi@msu.edu)>)

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Flavonoids are an extensive group of polyphenolic compounds that are found in almost every plant. These compounds play a role in development, abiotic stress tolerance and defense against pathogens and insect herbivory. They are also considered one of the largest groups of phytonutrients, and demonstrated to have crucial health benefits, such as anti-inflammatory and anti-oxidant properties. The goal of our research is to utilize liquid chromatography-mass spectrometry (LC-MS) to analyze a variety of flavonoids in different maize genotypes across the Wisconsin Diversity Panel and the recent Expired Plant Variety Protection lines. This will enable us to compare flavonoid variety in approximately 800 maize genotypes, and investigate the genetic control of polymerization inhibition of polyphenolic compounds in mature maize kernels for an increased accumulation of bioavailable beneficial flavonoids in maize seeds.

P145 

## Isolation of mutant alleles for transcription factors with putative roles in the regulation of maize phenolic biosynthesis

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Transcription factors (TFs) represent only 5-10% of the maize genome, but in large part control transcriptional regulation of all protein-coding genes. In many cases, single TFs can regulate multiple structural genes within biosynthetic pathways, making them attractive targets for control of key metabolites. This project is focused on identification of TFs that regulate maize phenolic biosynthesis, as pathway enzymes are well-characterized and the specialized metabolites produced (general phenylpropanoids, flavonoids and lignins) are important for maize crop productivity. Prior research utilized a yeast one-hybrid screen to identify a set of 45 TFs that interact with promoters of multiple phenolic biosynthesis genes. We have collected putative loss-of-function alleles for these genes from available sequence indexed resources resulting in alleles for 25 of the 45 TF genes. In total, 64 different events were obtained and used for genotyping, back-crossing, and characterization. Many of these alleles (38/64) exhibit segregation for the predicted insertion and provide segregating alleles for 20 TF genes. Expression analysis confirms loss of transcript expression or altered transcript structure for many exon insertion events, but few alleles with UTR or intron insertions result in expression level or transcript structure changes. These mutant alleles provide the basis for analyzing the role of these TFs in regulation of gene expression and phenolic metabolites.

Funding acknowledgement: National Science Foundation (NSF)

P146 

## Maize kernel development and fl2-RFP accumulation in response to variable nitrogen

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Nitrogen (N) fertilizers are a major pollutant and input cost of maize (*Zea mays*) production, but their negative effects can be mitigated through the development of cultivars with higher N use efficiency (NUE). Yield increases due to N fertilizers are primarily attributed to increases in kernel number, which is determined early in kernel development. Responses to N at this early stage of development are difficult to investigate, due to the complex path of N within the plant. *In vitro* kernel culturing, a method to control the supply of nutrients to the developing kernels, was performed on hybrid ears pollinated with a *floury2*-RFP transgenic line encoding the zein storage protein fused to RFP. Image analysis of samples show that culture N and genotype had a significant effect on RFP measures, supporting the view that N availability to developing kernels rather than vegetative N dictates zein accumulation. In previous experiments, B73 X Mo17 kernels were assayed by RNA sequencing and metabolite profiling of free amino acids. Trait and gene expression data were integrated to create coexpression networks. Multiple networks were created with a subset of samples holding one treatment condition constant in addition to creating a network utilizing all samples. Components of the Brassinosteroid biosynthesis pathway were downregulated by culture N early in development but upregulated at later timepoints. Additionally, *floury3*, a PLATZ transcription factor known to be involved in endosperm development, consistently increased throughout development, but the magnitude of this response was greatly affected by field N levels and not by nutrient supply *in vitro*. These findings demonstrate that *in vitro* kernel culturing provides the ability to separate early and late N supply, which leads to the identification of candidate genes in complex pathways, such as development and N metabolism.

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

P147

## Maize root diterpenoids influence the root-associated microbiome

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Plants deploy specialized metabolites to communicate with other organisms and cope with environmental challenges. This includes interactions with microbial communities, in which plants exchange sugars for available nutrients as well as protection against environmental stressors, as well as pathogenic microbes. Here, we report that maize root diterpenes - a group of specialized metabolites with versatile functions in stress resilience - influence rhizosphere bacterial communities. Distinct from the gibberellin biosynthesis pathway, both kauralexins and dolabralalexins are synthesized via the copalyl diphosphate synthase, ZmAn2, before branching into separate pathways. The *an2* (*anther ear 2*) maize mutant is deficient in forming both kauralexin and dolabralalexin metabolites, and exhibits enhanced stress susceptibility. Using 16S rRNA sequencing, we determined the bacterial community compositions of the *an2* mutant compared to its wild type sibling. Under well-watered conditions, distinct bacterial communities and diversities were observed between mutant and wild type plants, whereas the microbiome compositions became indistinguishable under drought conditions. These findings suggest that diterpenes play an important role in shaping the rhizosphere microbiome, while alternate mechanisms may be dominant under drought stress.

Gene / Gene Models described: *an2*; Zm00001d029648

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

P148 

## Maize transformation – applications, tools and availability

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The demand for efficient maize transformation is rising in the research community. The successful use of CRISPR/Cas9-mediated gene editing and affinity purification – mass spectrometry (AP-MS) in maize is opening new possibilities to test hypotheses in this model crop. Plant transformation and regeneration remain however bottlenecks.

Here we report on CRISPR/Cas9-mediated gene editing to create loss-of-function alleles in the maize inbred line B104 using Agrobacterium-mediated transformation and in H99 using biolistics. We describe the use of visual marker genes such as VIRESCENT YELLOW-LIKE (VYL) that when knocked-out lead to pale-yellow phenotypes that are already distinguishable in T0 plants during regeneration. Using VYL as a marker, we examined transient co-transformation of the morphogenic regulators OVULE DEVELOPMENT PROTEIN2 (ODP2/BBM) and WUSCHEL2 (WUS2) with a T-DNA of interest to boost maize transformation. Using this co-transformation method, we were able to create inherited alleles for VYL in B104.

Maize transformation is also used to express affinity-tagged proteins in vivo allowing purification and identification of interactors by mass-spectrometry. This has been successfully performed using leaf material from transgenic plants (Nelissen et al., 2015). Here we will present a new approach in which transgenic embryogenic callus can be used for AP-MS of widely expressed protein complexes. Within 6 weeks after transformation and starting with only 90 immature embryos, we could obtain enough biomass for AP-MS and successful identification of interactors (Bontinck et al., in preparation).

The VIB Crop Genome Engineering Facility provides researchers easy access to a maize transformation and gene-editing service based on the classic protocols for B104 transformation (Frame et al., 2002, Coussens et al., 2012). We have serviced several European groups and companies the past two years. For more information, visit our website ([www.psb.ugent.be/cgef](http://www.psb.ugent.be/cgef)) or email [lapau\(at\)psb.ugent.be](mailto:lapau(at)psb.ugent.be).

The VIB AP-MS Platform offers the plant research community access to a protein complex purification platform. For more information, visit our website (<https://www.psb.ugent.be/tap-platform>) or email [gejae\(at\)psb.ugent.be](mailto:gejae(at)psb.ugent.be).

Gene / Gene Models described: *ODP2*, *WUS2*; GRMZM2G141638, GRMZM2G028622

P149

## Mapping of a modifier that confers resistance to damping-off disease in JA-deficient maize mutant

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Oxo-Phytodieneic acid reductases (OPRs) are important enzymes in the synthesis of jasmonic acid (JA). JA-deficiency results in complete lack of immunity to *Pythium* spp., the causal agent of damping-off disease. For this reason, JA-deficient mutants are unable to survive in non-sterile soil. In maize mutation of the genes *opr7*; *opr8* is sufficient to block JA synthesis, these mutants are characterized by the formation of a feminized tassel structure (tasselseed) phenotype and are unable to grow under field conditions in both B73 and CML176 genetic backgrounds. Single *opr7* or *opr8* mutants have normal phenotypes. Surprisingly, we have found that when introgressed into the W438 background, *opr7*; *opr8* double mutants display the tasselseed phenotype characteristic of JA deficiency but survive soil-borne pathogens and are viable in the field. This suggested that W438 encodes a modifier that enables survival while conserving a tasselseed phenotype. Here, we present progress in the mapping of this modifier locus that acts to suppress the pathogen susceptibility phenotype of *opr7*; *opr8* mutants in the W438 background.

We crossed a tasselseed individual in the W438 background (*opr7/opr7*; *opr8/opr8*) with a phenotypically wild-type *opr8/opr8*; *Opr7/opr7* individual in the B73 background. Individuals genotyped as *opr8/opr8*; *Opr7/opr7* from the resulting F1 progeny were self-pollinated, resulting in three phenotypic classes in the F2: tasselseed, dead, and normal plants. The phenotypic ratio in the F2 was consistent with the action of a single dominant modifier locus inherited from the W438 background. One hundred ninety-two plants from F2 were sampled and will be genotyped to identify the genomic region involved in the production of tasselseed plants with normal plants by GBS. In order to increase the resolution of the possible QTL we made more backcrosses using tasselseed plants pollinated with B73 pollen. These experiments will enable the identification of the region carrying the modifier and fine mapping the responsible locus.

Gene / Gene Models described: *opr7*, *opr8*; GRMZM2G148281, Zm00001d050107

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P150

## Mapping protein interactions in the *Zea mays* kinetochore

(submitted by AB Abera <[aabera@hamilton.edu](mailto:aabera@hamilton.edu)>)

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Kinetochores are protein structures assembled on the centromeric region of chromosomes and play a major role in cell division. There are four proteins that have been identified in the *Zea mays* kinetochore: CENH3, CENPC, NDC80, and MIS12 (MIS12-1 and MIS12-2). These proteins are conserved throughout most organisms, from plants to humans. CENH3 and CENPC are located in the inner kinetochore, associated with centromeric DNA, while NDC80 and MIS12 are located in the outer kinetochore, attached to the spindle. The goal of our study was to analyze the interactions between the four maize kinetochore proteins. In order to investigate these interactions, we used a yeast two-hybrid assay, which detects protein interaction through activation of a selectable yeast reporter genes. We have found that MIS12-2 interacts with itself in a dimerization event, and MIS12-2 interacts with CENPC. We are mapping the domains responsible for these interactions. Understanding how individual proteins interact will inform how the larger kinetochore structure is created and aids chromosome segregation.

Gene / Gene Models described: *CENH3*, *CENPC*, *MIS12-1*, *MIS12-2*, *NDC80*; GRMZM2G158526, GRMZM2G114315, Zm00001d026640, Zm00001d001798, Zm00001d032029

P151

## Mechanisms of resistance for Goss's wilt of maize

(submitted by Alexander Mullens <[mullens3@illinois.edu](mailto:mullens3@illinois.edu)>)

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The vascular pathogen *Clavibacter nebraskensis* (*Cn*) causes Goss's wilt and blight of maize. In susceptible maize varieties it causes foliar blight lesions and, in severe cases, vascular wilt. Systemic wilt symptoms are most often observed in young plants and are characterized by a slimy water-soaked appearance of the stalk accompanied by orange discoloration. Leaf blight symptoms are more commonly observed in mature plants and are characterized by long tan lesions with small dark water-soaked spots commonly referred to as freckles. Goss's wilt is an important disease in the US and Canada, and this pathosystem can serve as a model for how gram-positive bacterial pathogens interact with monocots. We hypothesize that colonization and movement of *Cn* differs between resistant and susceptible maize lines. In order to test this hypothesis, we implemented a strategy to visually track bacterial growth in planta. We developed a protocol to transform *Cn*, which we used to generate green fluorescent protein (GFP)-labeled *Cn* strains to study the colonization and movement of the bacteria in the plant. To our knowledge this is the first time *Cn* has been transformed to express GFP. Experiments to evaluate the pathogenicity and stability of the GFP-labeled strains will be conducted. We will use the GFP-labeled strains to study the mechanisms of resistance in maize to Goss's wilt.

Funding acknowledgement: University of Illinois

P152

## Metabolomic analysis of maize nodal root growth under precisely controlled water deficit

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In maize and other cereals, most water is acquired by whorls of nodal roots that develop sequentially from subterranean stem nodes rather than by seedling (primary and seminal) roots. Nodal roots frequently must emerge and elongate through very dry topsoil to access water at depth. The molecular genetic mechanisms of nodal root elongation maintenance at tissue water potentials that inhibit leaf and stem growth remain largely uncharacterized. We utilized a split-root system to separately impose precise, constant soil water potentials on seedling and nodal roots of the maize inbred line FR697. A water deficit regime was designed to simulate field drought conditions with dry topsoil surrounding the nodal roots and greater water availability within the seedling root compartment. Under these conditions, FR697 exhibited a classical drought stress phenotype, including reduced leaf expansion, an increased root:shoot biomass ratio, and osmotic adjustment within the nodal root tips. Importantly, node 2 root elongation was completely maintained, in contrast to both node 1 and seedling primary roots. To investigate the molecular basis of root growth maintenance under water deficit, kinematically defined regions of the root growth zone comprising 1) meristematic, 2) rapidly elongating, and 3) decelerating tissues were harvested from water deficit-treated and control plants. Soluble metabolomes were generated from each of these tissues. Surprisingly, metabolomic responses to water deficit were broadly conserved between node 2 roots and comparable regions of the seedling primary root tip despite the large observed difference in growth maintenance. Efforts are underway to integrate these data with transcriptomic and proteomic analyses of equivalent tissue samples to refine our understanding of both conserved and unique responses of nodal root tips to water deficit relative to the seedling primary root. Novel candidate genes arising from these multi-omic analyses will be selected for targeted mutagenesis and analyzed alongside current candidates involved in carbohydrate transport and metabolism.

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



P153 

## **Metabolomic profiling of root and exudates samples from maize**

(submitted by Camila Pereira Braga <[braga\\_ca@unl.edu](mailto:braga_ca@unl.edu)>)

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Integrated analytical platform for global metabolic profiling was used to study the metabolism of plant roots and exudates. The platform consists of several steps including sample preparation, metabolite extraction and separation by various LC-MS techniques. To maximize coverage of metabolites for profiling analysis, liquid-liquid extraction by methanol/water/chloroform was used to separate polar (aqueous phase) and non-polar (organic phase) molecules. For root's samples, tissue was homogenized in MetOH at concentration 20 mg/mL and total amount of 40 mg and 4 mg of homogenized tissue was evaluated for number and quality of identified features (peaks representing potential metabolites). For exudates, 150 mL of samples was collected, lyophilized under vacuum and solubilized in 2 mL of water. The samples (1 mL) were extracted by methanol/water/chloroform by the same procedures as described for root's samples. The non-polar metabolites were analyzed using an Agilent 1200 Series HPLC coupled to a high-resolution Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS). The polar metabolites were analyzed using an UPLC M-Class System coupled to a Xevo G2-XS QTOF mass spectrometer. To identify major metabolic differences, selected genotypes and controls were compared using hierarchical clustering. To map the interaction between root and its environment, both common and unique metabolites in the exudates and roots were quantified and preliminarily identified. For root samples, 3,043 features (811 tentatively identified) were detected in non-polar fraction, and 2,557 (216 tentatively identified) in polar fraction. For exudates, 763 features (219 tentatively identified) were detected in non-polar fraction, and 783 (105 tentatively identified) in polar fraction.

Funding acknowledgement: National Science Foundation EPSCoR Center for Root and Rhizobiome Innovation Award OIA-1557417

P154 

## **Microbiomes of plant-mutualistic mycorrhizal fungi as a model for multi-partner interactions belowground**

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Plants associate with mutualistic mycorrhizal fungi, and those fungi in-turn associate with a specific set of microorganisms. Together, the community of host-associated microbes is known as the microbiome, and it can interact with the host across the symbiotic spectrum from negative to positive. While the interactions between plant roots and their microbes are an active field of study, the interactions between mycorrhizal fungi and their microbiomes are largely unknown. Here, I describe the microbiomes of a suite of mycorrhizal fungi sampled over a 3-year period in northern California by using high-throughput sequencing to identify the members of these microbiomes. I will discuss microbiome community structure across time and space, as well as how trophic modes and predicted biochemical pathways may explain how these microbes interact with their plant and fungal hosts in the rhizosphere environment.

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

P155 

## Multiple genes recruited from hormone pathways partition maize defenses

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Duplication and divergence of essential primary pathway genes underlay the evolutionary expansion of plant specialized metabolism; however, mechanisms partitioning parallel hormone and defense pathways often remain speculative. For example, the primary pathway precursor ent-kaurene is required for gibberellin biosynthesis and is likewise a proposed intermediate for maize kauralexin antibiotics. By integrating transcriptional co-regulation patterns, Genome Wide Association Mapping Studies, combinatorial enzyme assays, proteomics and targeted mutant analysis, we show that maize kauralexin biosynthesis instead proceeds via the positional isomer ent-isokaurene formed by a diterpene synthase pair recruited from gibberellin metabolism. The oxygenation and subsequent desaturation of ent-isokaurene by three promiscuous Cytochrome P450s and a novel steroid 5 $\alpha$  reductase indirectly yields predominant ent-kaurene-associated antibiotics required for *Fusarium* stalk rot resistance. The divergence and differential expression of pathway branches derived from multiple duplicated hormone-metabolic genes minimizes dysregulation of primary metabolism via the circuitous biosynthesis of ent-kaurene-related antibiotics that avoids large-scale production of growth hormone precursors during pathogen defense.

Funding acknowledgement: National Science Foundation (NSF)



P156 

## Multiplex genome editing in the Illinois Long Term Selection experiment

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The Illinois Long Term Selection Experiment (ILTSE) is a unique germplasm resource for studies of genome evolution and genetic variants that contribute to phenotypic traits. Moreover, some of the ILTSE genotypes create highly regenerable embryogenic type I callus, which enables transformation and genome editing approaches to characterize gene function. Here, we describe results from site-specific mutagenesis by CRISPR-Cas9 in Illinois Low Protein (ILP). We have initially targeted two genes of interest: Lemon White 1 (*Lw1*) and L-Asparaginase (*ASNase*). The *Lw1* experiments were performed as a proof of concept to generate albino plants easily detectable in a population of regenerated plants, while *ASNase* experiments attempt to validate the contribution of this gene to the phenotypic variation for grain protein concentration observed in the ILTSE. For each gene, we designed multiple guide RNAs and tested their function using an in vitro Cas9 cleavage assay. Plasmids were created where Cas9 and ribonuclease Csy4 were controlled by the maize ubiquitin 1 promoter and an array of four guide RNAs separated by Csy4 spacers were expressed as a single transcript driven by the strong viral Cestrum (*CmYLCV*) promoter. This strategy allows us to minimize plasmid size and for multiplexed guideRNAs. Vectors were delivered to embryogenic calli using biolistics in the absence of morphogenic regulators, and transgenic events selected. Multiple mutant albino plants indicative of biallelic mutations were recovered from the *Lw1* experiment in both the ILP1 and H99 control genotypes at ~2% efficiency. DNA sequencing demonstrated the creation of various types of mutant alleles. Screening of *ASNase* events is ongoing with a population of over 100 regenerated plants. Our experiments demonstrate the first CRISPR-Cas9-Csy4 multiplexed editing in maize, which serves as a platform for continuing experiments in studying kernel composition and nitrogen use in the ILTSE.

Gene / Gene Models described: *Lw1*, *ASNase*; Zm00001d033896, Zm00001d002052

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

P157

## Network analyses shed light on the dynamics of cuticle development in the maize leaf

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Plant cuticles are composed of wax and cutin, and evolved in the land plants as a hydrophobic boundary between the plant epidermis and the environment to reduce water loss. The expanding maize adult leaf harbors a dynamic biochemical gradient of cuticle components from leaf base to tip. Laser microdissection RNA Sequencing (LM RNA-Seq) was performed along this leaf proximodistal gradient, and network analysis identified regulators of cuticle biosynthesis and deposition. Correlations between cuticle development and cell wall biosynthesis are identified, as well as roles for auxin and brassinosteroids. In addition, our network analyses revealed that light signaling via PHYTOCHROME B (PHY B) specifically impacts the deposition of longer chain wax compounds in the mature cuticle. Transcriptomic analysis of light/dark exposed plants suggests that a specific LIPID TRANSFER PROTEIN (LTP), activated by PHY B signaling, is responsible for the abnormal wax phenotype in phy B1 phy B2 double mutants. We propose that light perception and signaling played a critical role in the regulation of cuticle biosynthesis and deposition during the evolution of land plants.

Funding acknowledgement: National Science Foundation (NSF)

P158 

## One-step genome editing of elite crop germplasm during haploid induction

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Genome editing using CRISPR-Cas9 works efficiently in plant cells but delivery of genome editing machinery into the vast majority of crop varieties is not possible using established methods. We co-opted the aberrant reproductive process of haploid induction (HI) to induce edits in nascent seeds of diverse monocot and dicot species. Our method, named “HI-Edit”, enables direct genomic modification of commercial crop varieties. HI-edit was tested in field and sweet corn using a native haploid inducer line, and extended to dicots using an engineered CENH3 HI system. We also recovered edited wheat embryos using Cas9 delivered by maize pollen. Our data indicate that a transient hybrid state precedes uniparental chromosome elimination in maize HI. Edited haploid plants lack both the haploid inducer parental DNA and the editing machinery. Therefore, edited plants could be used in trait testing and directly integrated into commercial variety development.

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## Pentatricopeptide repeat protein DEFECTIVE KERNEL 40 is essential for post-transcriptional processing of *cox3*, *nad2* and *nad5* transcripts and is required for mitochondrial function and kernel development in maize

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Pentatricopeptide repeat (PPR) proteins play important roles in RNA editing in both mitochondria and plastids as site-specific recognition factors. However, the mechanism underlying E+ subgroup PPR proteins on RNA editing remains elusive. Here, we isolated a maize mutant, *defective kernel 40* (*dek40*). Positional cloning, genetic and molecular analyses revealed that DEK40 encode a new E+ subgroup PPR protein that localized in the mitochondrion. DEK40 recognizes and directly binds to *cox3*, *nad2* and *nad5* transcripts and functions on editing of them. In *DEK40* loss function mutant, abolished the C-to-U editing of *cox3*-314, *nad2*-26 and *nad5*-1916 leads to accumulated reactive oxygen species and promoted programmed cell death in endosperm cells due to the dysfunction of mitochondrial complex I and IV. Gene expression profiling showed that some biosynthesis and metabolism pathway genes were altered in *dek40* mutant compared with wild-type control, including glutathione metabolism pathway and starch biosynthesis pathway, results in abnormal development of the maize mutant kernels. Taken together, our results provide the solid evidences on the molecular mechanism underlying DEK40 on RNA editing, and extends our understanding of PPR E+ type protein in editing function and kernel development in maize.

Key words: kernel development; maize; mitochondrion; pentatricopeptide repeat protein; RNA editing

Funding acknowledgement: National Natural Science Foundation of China (91535109 and 91735301), the National Plant Transgenic Program (2016ZX08003-003), Taishan Scholars Project (ts201712024).

P160

## **Perturbation of the C terminus of ZmSDW3 alters plant architecture in maize (*Zea mays* L.)**

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Plant height and leaf angle are two crucial determinants of plant architecture in maize and are closely related to lodging resistance and canopy photosynthesis at high planting density. These two traits are primarily regulated by phytohormones, including gibberellins, brassinosteroids, and auxin. Here, we characterized a semidominant maize mutant, *Semi-dwarf3* (*Sdw3*), which exhibits shorter stature and larger leaf angle than the wild type. Scanning electron microscopy observation showed that inhibition of longitudinal cell elongation in the internode and promotion in the auricle were mainly responsible for reduced plant height and enlarged leaf angle in *Sdw3*. Through map-based cloning, we identified a transposable element insertion in the candidate gene *ZmSDW3*. The transposon alters the C terminus of *ZmSDW3*. Transgenic analysis confirmed that the mutant *ZmSDW3* gene confers the phenotypes of *Sdw3*. Enzyme activity and protein degradation assays indicated that the altered C terminus of *ZmSDW3* in *Sdw3* increases this protein's stability but does not affect its catalytic activity. In addition, we demonstrated that *ZmSDW3* plays crucial roles in root development, flowering time, and leaf number, indicating that *ZmSDW3* is an important gene with pleiotropic effects during maize growth and development.

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

P161

## **Polymerase Chain Reaction primers that identify the *Ae1-5180* allele**

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Starch is composed of less branched amylose and highly branched amylopectin. The branching in starch formation is catalyzed by Starch Branching Enzymes. In maize, starch branching enzyme IIB (SBEIIB) is the primary starch branching enzyme in the endosperm and is encoded by *ae1* (*amylose extender 1*). SBEIIB catalyzes the cleavage of an  $\alpha$ -1,4 glycosidic bond and replacing it with an  $\alpha$ -1,6 glycosidic bond, therefore resulting in a high proportion of amylopectin. The proportion of amylopectin is about 75% in common Mid-Western Dents. An antimorph *Ae1-5180* had been identified that acts in a dominant fashion to eliminate SBEIIB enzyme in maize endosperm, which decreases the proportion of amylopectin. As a result, the kernels appear glassier and sometimes more misshapen. The cause of mutation was described as the insertion of *Mu1* (*Mutator 1*) transposon on functional *ae1* gene. Based on the restriction map published in the 1980s, we developed a predicted sequence for *Ae1-5180* mutant allele using the sequences of the *ae1* gene and *Mu1* transposon. Polymerase chain reaction (PCR) primers were developed using the predicted sequence for *Ae1-5180*. The primers were tested on samples expected to have the *Ae1-5180* allele. H99ae, which is homozygous for the recessive *ae1* allele and B73, which possesses the functional *Ae1* allele, were used as controls. The sample that possessed *Ae1-5180* produced a 600bp band. The resulting band was sequenced and showed a 100% match with *Mu1* transposon for 104 bp and 99% with *ae1* gene for 438 bp.

Funding acknowledgement: SDSU Agricultural Experiment Station

P162

## Root exudates from young maize inbreds exposed to cold stress analyzed by NMR and metabolomic analyses

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Young maize plants in the field experience a multitude of stressors. One such stressor, cold, is especially relevant if planting occurs earlier in the season. However, early planting is a strategy farmers can use to improve their yields. Root exudates are excreted in part to communicate with microbes in the rhizosphere. In the case of cold stress, microbes recruited to the root can produce a protective layer that make the plant more resistant. Therefore, in the event of cold stress, the plant may change the exudates it typically releases from its roots. In maize inbreds that are considered to be cold resistant, patterns of exudation may be observed to differ from those of susceptible inbreds. This is particularly interesting because if there is a relationship between a genotype's ability to withstand the cold and the exudates that it excretes, sampling exudation patterns in early development could be used to predict how the stress would affect a plant as an adult. In 2016, over 351 exudate samples from 11 maize inbreds grown in cold or control conditions were extracted and frozen. For each genotype, between 14 and 47 spectra were collected by NMR (nuclear magnetic resonance) spectroscopy. The spectra were processed in ChemoSpec and analyzed using MetaboAnalyst. A series of statistical analyses were conducted to find peaks that significantly differed between genotypes and between cold and control conditions.

Funding acknowledgement: National Science Foundation (NSF)

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## Setaria viridis as a model for translational genetic studies of jasmonic acid-related insect defenses in *Zea mays*

(submitted by Charles Hunter <[charles.hunter@ars.usda.gov](mailto:charles.hunter@ars.usda.gov)>)

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Little is known regarding insect defense pathways in *Setaria viridis* (*Setaria*), a model system for panicoid grasses, including *Zea mays* (maize). It is thus of great interest to compare insect herbivory responses of *setaria* and maize. Here we use metabolic, phylogenetic, and gene expression analyses to measure a subset of jasmonic acid (JA)-related defense responses to leaf-chewing caterpillars. Phylogenetic comparisons of known defense-related maize genes were used to identify putative orthologs in *setaria*, and candidates were tested by quantitative PCR to determine transcriptional responses to insect challenge. Our findings show that while much of the core JA-related metabolic and genetic responses appear broadly conserved between *setaria* and maize, production of downstream secondary metabolites such as benzoxazinoids and herbivore-induced plant volatiles are dissimilar. This diversity of chemical defenses and gene families involved in secondary metabolism among grasses presents new opportunities for cross species engineering. The high degree of genetic similarity and ease of orthologous gene identification between *setaria* and maize make *setaria* an excellent species for translational genetic studies, but the species specificity of downstream insect defense chemistry makes some pathways unamenable to cross-species comparisons.

Funding acknowledgement: United States Department of Agriculture (USDA)

P164

### **Single gametophyte sequencing reveals that crossover events differ between sexes in maize**

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Meiotic crossover (CO) plays a key role in producing gametophytes and generating genetic variation. The patterns of CO production differ inter- and intra-species, as well as between sexes. However, sex-specific patterns of CO production have not been accurately profiled independently of genetic backgrounds in maize. Here, we develop a method to isolate single female gametophyte for genomes sequencing in maize. We show that more COs are observed in male (19.3 per microspore) than in female (12.4 per embryo sac). Based on Beam-Film model, the more designated class I and II COs are identified in male than in female. In addition, CO maturation inefficiency (CMI) is detected in some of the genetic backgrounds, suggesting that maize may be an ideal model for dissecting CMI. This research provides insights toward understanding the molecular mechanism of CO production between sexes and may help to improve maize breeding efficiency through paternal selection.

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P165

### **Site-specific recombinases: Tools for genetic engineering in maize**

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Site-specific recombinases activate strand-switching reactions between two recombinase binding sites (RBS) in a targeted and precise manner. Depending on RBS positioning and orientation, reactions result in the integration, excision, or inversion of specific DNA sequences. If recombinase technology is coupled with conventional genetic engineering methods in plants, recombinases could possibly increase the efficiency of the transformation process by eliminating random transgene incorporation, targeting genes of interest to specific locations in the maize genome with predictable expression patterns. Additionally, recombinases could give researchers more control over established transgenic lines by enabling the removal of selectable markers through breeding strategies, a process that will subsequently allow gene stacking using a single selectable marker. Using DsRed expression constructs, this project demonstrates functionality of a collection of different recombinase enzymes in maize (Cre, R, Flpe, PhiC31 Integrase, and PhiC31 Excisionase). Recombinase technology will serve as the framework for future genome engineering projects, including targeted transgene integrations and engineered minichromosomes.

Funding acknowledgement: National Science Foundation (NSF)

P166

## Spatial-temporal analysis of cuticular lipid compositions reveals genotype-specific responses to emergence from husk leaves

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A hydrophobic cuticle covers most plant surfaces and provides the first layer of defense from both abiotic and biotic environmental stresses. Cuticular lipids that accumulate on maize silks consist of mostly hydrocarbons and minor amounts of fatty acids (16 to 33 carbons in length), which are products and precursors of the hydrocarbon biosynthetic pathway, respectively. Previous work in inbred B73 has shown a 3- to 5-fold increase in hydrocarbon accumulation on silks that have emerged into the external environment as compared to the silks that remain encased by husk-leaves. Moreover, cuticular hydrocarbon load is 3-fold higher on emerged silks from B73 compared to inbred Mo17. However, it is unclear whether prior to silk emergence cuticular lipid accumulation is dynamic along the length of the silks or between genotypes. To assess this, we profiled cuticular lipids along the silk length from B73 and Mo17 at two different growth stages, either on the day silks would first emerge or three days after silks had emerged from the husk leaves. Silks were sectioned into three contiguous segments for encased tissues and two segments for emerged tissues, cuticular lipids were extracted, and then characterized via gas chromatography-flame ionization detection. We observe that cuticular lipid accumulation differs along the lengths of the silks, both at 0 days and at 3 days post-silk emergence (PSE). Although total accumulation is similar in encased tissues at 0 and 3 days PSE, the composition of the metabolome differs. For example, the relative abundance of fatty acids is lower in encased tissues at 3 days PSE, especially in inbred B73, which accumulates twice the amount of hydrocarbons products on emerged silks as compared to Mo17. This study will provide additional insights into product-precursor relationships within the metabolic network for cuticular lipid accumulation.

Funding acknowledgement: National Science Foundation (NSF), Division of Integrative Organismal Systems

P167

## Teff $\alpha$ -globulins in maize endosperm cells mimic wheat HMW glutenins at the molecular and cellular level

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Today gluten-free foods have become essential in groceries and restaurants due to increased gluten sensitivity throughout the world. A new alternative to gluten-free wheat could be teff (*Eragrostis tef*), a grain consumed in Ethiopia. Its evolutionary distance is reflected in teff's composition of storage proteins, which resembles that of maize except for the higher levels of  $\alpha$ -globulins that are close to the wheat HMW glutenins. If we were to add teff  $\alpha$ -globulins to maize, could this reverse the evolutionary distance between maize and wheat seed proteins? Indeed, we could find evidence about the convergent evolution of teff  $\alpha$ -globulins and wheat HMW glutenins at the molecular and cellular levels. The teff  $\alpha$ -globulin Etglo3 can form polymers through intermolecular disulfide bonds in maize, like wheat gluten under non-reducing condition. Etglo3 also triggers the formation of a novel protein storage vacuole (PSV) in maize starchy endosperm cells. Immuno-gold labeling indicates that Etglo3 is almost exclusively deposited in the CSV as electron-dense inclusion. Its morphology has similarity with wheat protein storage vacuoles, which are large, amorphous and electron-dense inclusion within them. The Y2H (yeast-two-hybrid) and BiFC (bimolecular fluorescence complementation) results confirm interaction of Etglo3 with all-type zeins, suggesting that teff  $\alpha$ -globulins is molecularly functional for prolamins interactions. Furthermore, crossing Etglo3 transgene with RNAi lines of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -zeins reveals that the expression and accumulation of Etglo3 is dramatically reduced in the background of  $\gamma$ -zein RNAi. Immuno-gold labeling further shows that  $\gamma$ -zeins, but not  $\alpha$ -zein, exists in the electron-dense inclusion, suggesting that 27-kDa  $\gamma$ -zein is also involved in the formation of CSVs. This study demonstrates that teff  $\alpha$ -globulins could functionally modify the property of maize storage proteins, thereby providing a potential strategy for future engineering maize to make gluten-free bread.

Funding acknowledgement: The Selman A. Waksman Chair in Molecular Genetics



P168

**The high expression of Rubisco subunits with its assembly factor RAF1 does not confer a dramatic metabolic load during drought stress, in comparison to control or transgenic WT-level Rubisco plants, however it highly contributes to its recovery**

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Finding ways to increase Ribulose-1,5-bisphosphate carboxylate/oxygenase (Rubisco) activity in crops to improve photosynthesis has long been a key objective in efforts to increase production. We have recently shown that overexpressing Rubisco subunits along with assembly factors in maize resulted in higher Rubisco content, which was associated with higher photosynthetic capacity and dry biomass. The question remains, however, whether the high expression level of these transgenes would constitute a metabolic load that might be detrimental under environmental stress. In the present study, control and transgenic maize lines either overexpressing the Rubisco assembly factor 1 (RAF1), both large and small subunits of Rubisco (LSSS), or the two subunits along with their assembly factor (RAF1-LSSS), were subjected to terminal drought conditions to investigate the potential effects of metabolic load and the introduced Rubisco traits. The results presented here demonstrate that RAF1-LSSS line, which has increased Rubisco abundance relative to the other lines, was more affected than both WT and WT-level Rubisco lines in terms of gas exchange and plant growth parameters after 14 days of water stress. These differences were only minor, however, as both control and transgenic lines displayed severe growth delay and a dramatic drop in photosynthetic capacity. After returning plants to well-watered conditions, however, RAF1-LSSS displayed a more robust recovery based on photosynthesis, growth parameters, and dry root and shoot biomass compared to all other genotypes. We attribute this to its high Rubisco content and low MDA content measured prior to recovery. In addition, strong recovery was observed in RAF1 line, evidenced in higher photosynthetic rate and increased dry root and shoot biomasses than the control, differences that were not observed in non-stressed plants. Taken together, our results suggest that while the high-Rubisco trait is neutral during water stress, recovery is more rapid once the stress is removed.



P169

**The neomorphic *Carbohydrate partitioning defective1* mutant exhibits polarly localized ectopic callose deposition in root phloem in *Zea mays***

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Plants synthesize carbohydrates in photosynthetic source tissues and transport them, predominantly in the form of sucrose, to non-photosynthetic sink tissues to sustain their growth and development. This process, termed carbohydrate partitioning, is well characterized from anatomical, biochemical, and physiological perspectives while little is known concerning its genetic control. We have previously characterized the semi-dominant maize mutant *Carbohydrate partitioning defective1* (*Cpd1*), which exhibits ectopic callose deposition in the phloem of leaf veins, inhibiting sucrose export and resulting in small chlorotic plants that hyper-accumulate carbohydrates in their leaves. *Cpd1* mutants have diminished root mass and exhibit ectopic callose deposits in their root phloem. In *Cpd1/Cpd1* homozygous mutant plants this deposition occurs throughout the root; however, in *Cpd1/+* heterozygous mutant plants it occurs in a polar manner at the site of lateral root formation. This novel phenotype does not occur in mutants with inhibited root development, such as *barren inflorescence2* and *vanishing tassel2*. Due to *Cpd1* being a semi-dominant mutation, chromosome dosage studies were performed to identify if *Cpd1* is a gain- or loss-of-function mutation. Karyotypes of plants expressing the *Cpd1/+* mutant phenotype were generated using fluorescent in situ hybridization (FISH), and several hyperploid mutant individuals were identified. These results suggest that *Cpd1* is a neomorphic gain-of-function mutation as a 1:2 dosage of the mutant to wild-type allele confers the same phenotype as a 1:1 dosage. Transgenic plant studies are in progress to identify the causative mutation by independently adding the mutated coding sequence from three candidate genes driven by their native promoters. Additionally, GUS-YFP reporter constructs driven by the native promoters are being transformed into maize to determine each candidate's tissue-specific expression profiles. This work will identify the causative mutation resulting in the *Cpd1* phenotype and contribute unique insights into phloem development, underpinning whole-plant carbohydrate partitioning.

Funding acknowledgement: National Science Foundation (NSF)

P170 

## The role of phospholipid balance in maize adaptation to highlands

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After domestication from lowland teosinte in the warm, humid Mexican southwest, maize colonized the highlands of Mexico and South America. In the highlands, maize was exposed to a whole range of environmental factors that differ from the site of domestication, including, among others, lower temperatures, soils with lower phosphorus availability and different biological pressures. We hypothesize that glycerolipid metabolism remodeling was important in the process of maize adaptation to highlands.

I will show results from common garden experiments in Mexican lowland and highland common gardens where we grew maize mapping populations and using quantitative biochemical genetics tools we identified major QTLs that explain the conversion of phosphatidylcholines (PCs) to lyso-phosphatidylcholines (LPCs) leading to a high PC/LPC ratio that is particularly conserved in Mexican highland landraces. We have identified a couple of genes (ZmPla1.2 and ZmLpcat1) that code for enzymes controlling the PCs/LPCs ratio as the most likely causative genes of the PC/LPC conversion QTLs.

We then used GBS data from 3200 maize landraces and whole genome sequences from another 30 landraces across the Americas and identified SNPs within the coding regions of ZmPla1.2 and ZmLpcat1 that show clear signs of selection to highlands. Other genes controlling PCs/LPCs ratio were also found to be under selection in highland maize.

I will present ongoing work to identify the causative SNPs on candidate genes that lead to this biochemical phenotype, their natural variation and ultimately the physiological mechanisms that are affected by it and their possible adaptive significance in maize highland adaptation.

Gene / Gene Models described: ; GRMZM2G481755, GRMZM2G353444

Funding acknowledgement: National Science Foundation (NSF), Conacyt, Cinvestav

P171

## The SUMO ligase MMS21 is required for maize seed and plant development

(submitted by Junya Zhang <[zhangjunya@wustl.edu](mailto:zhangjunya@wustl.edu)>)

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Covalent conjugation of Small Ubiquitin-Like Modifier (SUMO) to various intracellular proteins is an essential post-translational modification in plants that provides protection against numerous biotic and abiotic challenges. Using an ATP-dependent E1→E2→E3 conjugation cascade reminiscent of ubiquitylation, SUMO addition is directed by a small set of SUMO ligases (E3s) to provide essential mechanisms for controlling the function, activity, location and/or half-life of the targets. METHYL METHANESULFONATE-SENSITIVE21 (MMS21) is a SUMO E3 ligase having critical roles in stress protection and DNA endoreduplication/repair. To explore the functions of the MMS21 in maize development and stress protection, we used UniformMu reverse genetic resources and CRISPR/Cas9 mutagenesis to generate multiple loss-of-function *mms21* alleles. Homozygous *mms21* mutants exhibit pleiotropic phenotypes, including reduced seed size, smaller plant stature and leaf dimensions, delayed flowering, and reduced fecundity. Different alleles of *mms21* mutant seeds show variable seed severities ranging from a slightly wrinkled, etched endosperm with a normal looking embryo, to a shrunken collapsed endosperm accompanied by a defective embryo. The *mms21* mutant seeds still germinate, albeit poorly. While *mms21* seedlings still trigger stress-induced SUMOylation, a number of prominent SUMO-modified proteins were absent, reduced, or upregulated, indicating that MMS21 controls the SUMOylation of a subset of targets. Our data provide genetic evidence for the importance of *Mms21* and SUMOylation in maize reproduction, and vegetative and seed morphology and development.

Gene / Gene Models described: *Mms21*; GRMZM2G022065

Funding acknowledgement: National Science Foundation (NSF)

P172 

## Uncovering new elements of iron homeostasis in grasses

(submitted by Stavroula Fili <[sfili@umass.edu](mailto:sfili@umass.edu)>)

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Iron (Fe) is a micronutrient essential for plant growth and development, involved in processes such as photosynthesis and respiration. Humans also need iron for essential biological processes; insufficient iron intake leads to iron deficiency anemia, a condition that affects about 25% of the world's population. Most staple crops such as maize and wheat, belong to the family of grasses that have evolved a chelator-based strategy (Strategy II) to acquire iron from the soil. Grasses secrete specific molecules called phytosiderophores (PS) into the rhizosphere through the Transporter of Mugineic Acid 1 (TOM1; YS3 in maize). PS bind and solubilize iron(III), and the PS-iron complexes are taken up by the plant through the Yellow Stripe1 (YS1) transporter. Identification of new elements involved in Strategy II is necessary to understand how grasses regulate iron uptake and translocation into different tissues. Elucidating these elements is also an important step towards the improvement of crops. We have used mutants impaired in iron homeostasis which present characteristic chlorosis due to lack of chlorophyll (interveinal yellowing). We screened stocks marked as "yellow-striped" from the Maize Genetics Cooperation Stock Center (MGCSC) for this iron deficiency phenotype. The chlorotic mutants were used for complementation tests with *ys1* and *ys3* to discover new genes potentially involved in iron homeostasis. We identified three mutants (*ys\**) that are not allelic to *ys1* or *ys3*, as well as not allelic to each other, suggesting that their phenotype could be caused by novel genes involved in iron homeostasis. All novel mutants have lower iron content than wild-type in their leaves, indicating that the yellow-striped phenotype is caused by iron deficiency. Finally, we crossed *ys\** mutants to wild-type plants and generated F2 mapping populations. One of the *ys\** mutants is currently being used for bulked segregant analysis using next-generation sequencing (BSA-seq).

Gene / Gene Models described: *ys1*, *ys3*; GRMZM2G156599, Zm00001d041111

P173 

## Using Arabidopsis as a proxy to assess the molecular basis of natural variation for Vitamin E in maize

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Tocopherols are fat-soluble antioxidants produced by all plants species and are essential nutrients in the human diet as vitamin E. The six core genes (*VitTaminE* loci 1 through 6) of the vitamin E biosynthetic pathway have been identified and characterized in many plant species but a comprehensive understanding of the control of this trait is still lacking. This is exemplified by the observation that ~75% of QTL affecting seed tocopherol levels in both Arabidopsis and maize lack *VTE* genes in their intervals<sup>1,2</sup> and define novel loci affecting the trait. In studying maize grain tocopherol levels in the NAM panel, six novel QTL affecting grain tocopherols were mapped to single candidate genes<sup>2</sup>. They include a fibrillin protein, glycolipid transfer protein, transport protein particle (TRAPP) protein homolog and PhD finger transcription factor. Three independent Mu-tagged alleles were identified for the fibrillin gene and in each, tocopherols and tocotrienols in mature grain were significantly decreased. Because promising mu-tagged lines were lacking for most of the other loci, we also assessed whether obvious Arabidopsis orthologs for the maize candidate genes existed, whether their knockout phenotypes affected tocopherols and if overexpression of their maize orthologs could functionally complement the Arabidopsis mutant. Arabidopsis orthologs were identified for three of the four genes and knockouts shown to impact tocopherol levels in Arabidopsis seed and/or leaf. For the TRAPP and fibrillin proteins, the maize orthologs complemented the Arabidopsis mutant, demonstrating functional equivalency between the two systems. Our data indicate that important functional information can be obtained for a significant portion of novel maize tocopherol loci by assessing their activities across the two organisms and is consistent with a significant portion of the tocopherol genetic architecture being conserved between dicots and monocots.

Gene / Gene Models described: *vte*, *trapp*; GRMZM2G039373, GRMZM2G031028, GRMZM2G128176, GRMZM2G060870

Funding acknowledgement: National Science Foundation (NSF)

P174

## Utilizing a dark fluorescent protein in FRET-based biosensors

(submitted by Micah Rambo <[rambome@whitman.edu](mailto:rambome@whitman.edu)>)

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The ability to measure when and where plant hormones are active in real time is a key step in understanding how they regulate diverse aspects of growth and development. A variety of fluorescent biosensors have been developed to monitor hormone signaling in plants, but are often slow to respond, irreversible or rely upon loss-of-signal. We are pursuing a novel way to measure hormone activity in real-time using a new FRET (Förster Resonance Energy Transfer) approach. We are utilizing a recently developed dark fluorescent protein known as ShadowY which forms a FRET pair with GFP. ShadowY absorbs GFP fluorescence but does not itself emit light, meaning that when FRET occurs it is measured as a decrease in GFP fluorescence. Conversely, if FRET is disrupted an increase in GFP signal will be observed. Thus, FRET detection requires measurement of only a single wavelength. We first set out to confirm that the GFP-ShadowY FRET pair is functional by testing it in yeast by either (1) fusing the FRET pair together with a simple linker or by (2) attaching each FRET protein to a homodimerization domain. Analysis of the fused FRET pair confirmed that GFP quenching occurred. Full characterization of the dimerization FRET pair has been hampered due to toxicity in the yeast that is likely the result of overexpression of the FRET proteins. Further experiments will attempt to mitigate the toxic effect of overexpression and refine the ratio of the expressed constructs. Ultimately, this novel FRET construct is intended for use within a single-wavelength, fast-acting biosensor to measure hormone dynamics in living plants.

Funding acknowledgement: National Science Foundation (NSF), Whitman College

P175 

## What is the role of the minor decarboxylation pathway in maize C<sub>4</sub> photosynthesis?

(submitted by Jennifer Arp <[jarp@danforthcenter.org](mailto:jarp@danforthcenter.org)>)

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Photosynthesis is a critical component of agricultural productivity that presents a great opportunity for genetic improvement. One way plants have improved photosynthesis is through carbon concentrating mechanisms; however, even these pathways could be fine-tuned for greater efficiency. C<sub>4</sub> photosynthesis utilizes a carbon concentrating mechanism achieved through complicated anatomical and metabolic pathway architecture to overcome inefficiencies caused by the oxygenation reaction of Rubisco and photorespiration. Maize is an important crop that uses C<sub>4</sub> photosynthesis and has excellent genetic resources. Maize uses a combination of two C<sub>4</sub> pathways—the major decarboxylase is NADP malic enzyme (NADP-ME), but as much as a quarter of flux has been shown to use the phosphoenolpyruvate carboxykinase (PEPCK) pathway. In this project, a transposon-based mutagenesis approach was taken to knock out components of the C<sub>4</sub> pathway in maize. The *pepck1::Ds* mutant was measured for photosynthetic parameters using gas exchange and <sup>13</sup>CO<sub>2</sub> labeling. The mutant was also grown in a nitrogen responsive field site in 2017 and 2018 to assess the transcriptional response, yield component traits, and nitrogen use efficiency. A double mutant knocking out components of both the NADP-ME and PEPCK pathways was generated, and was unable to grow after exhausting its seed reserves, showing the necessity of a C<sub>4</sub> concentrating mechanism in maize.

Gene / Gene Models described: ; GRMZM2G001696, GRMZM2G085019

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

P176 

## A greenhouse mesocosm system for integrated environmental sensing, grass root phenotyping, and new sensor development

(submitted by Tyler Dowd <[tdowd@danforthcenter.org](mailto:tdowd@danforthcenter.org)>)

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Current methods of root phenotyping can only obtain small, highly constrained, or incomplete sections of root systems and do not capture their true complexity. To facilitate the visualization and analysis of large, freely-grown root systems of crop plants in their entirety, mesocosm growth systems were developed in two sizes: 3x3x6 ft and 4x4x6 ft, with internal volumes of 45 ft<sup>3</sup> (1.27 m<sup>3</sup>) and 80 ft<sup>3</sup> (2.25 m<sup>3</sup>), respectively. PVC scaffolds support internal grids of taught fishing line that divide the entire growth volume into many 4 x 4 x 6 in rectangular boxes and hold the 3D root structure in place upon excavation. Following several months of growth, sorghum and switchgrass plants were harvested, and photogrammetry methods were used to construct 3D point-cloud models of entire root systems from which 3D metrics such as root system biomass, convex hull volume, and solidity as a function of depth were estimated. Sensor arrays monitoring growth media matric potential and temperature as well as CO<sub>2</sub> levels were buried in a grid formation at depths of 1.25, 2.75, & 4.25 ft to assess environmental fluxes at regular time intervals. Methods of 3D modelling and data visualization of these environmental fluxes were developed and co-registered with 3D root architectures to develop structural-functional relationships. The mesocosm system is flexible to experimentation and monitoring of multiple biotic and abiotic environmental conditions, and the capture of full-sized 3D root systems allows ground truthing and development of new sensors that image roots and their activity.

Funding acknowledgement: ARPA-e

P177 

## A male gametophyte-specific myosin is required for pollen tube germination but not for development of the pollen grain

(submitted by John Fowler <[fowlerj@science.oregonstate.edu](mailto:fowlerj@science.oregonstate.edu)>)

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The actin cytoskeleton enables pollen tube germination and growth, and thus is crucial for fertilization and seed development. We identified a potential component of this system, a myosin encoded by GRMZM2G435294/Zm00001d044819 (designated *ZmXI-11* by Wang et al. 2013, Myosin-9 by Gramene, and *myo9* hereafter), as highly and specifically expressed in mature pollen. This expression pattern, similar to that of the most closely related paralogs in Arabidopsis, led us to hypothesize that *myo9* provides an ancient conserved function, potentially in pollen tube growth. To test this hypothesis, we obtained the *myo9::Ds-B.W07.0227* insertion allele from the Vollbrecht AcDs collection, and confirmed its location in the 13th intron via sequencing. By PCR genotyping, *myo9::Ds* segregated at a Mendelian rate through the female, but showed a complete male-specific transmission defect (0 mutants/251 progeny), consistent with *myo9* providing a crucial function in the haploid gametophyte. Hypoploid *myo9::Ds/-* plants showed no obvious differences from sibling hypoploid *myo9+/-* plants, but did not produce seeds when outcrossed as a male, confirming that the *myo9::Ds* defect is pollen-specific and absolute. Pollen RT-PCR showed that the *Ds* insertion was associated with a reduction in MYO9-RNA levels relative to wild-type. Two excision alleles both reverted to enable transmission through male, proving that the insertion in *myo9* causes the defect. In liquid culture, pollen germination frequency from *myo9::Ds* heterozygotes was half that of wild-type siblings; furthermore, pollen from *myo9::Ds/-* plants never germinated, and the *myo9-rB27* revertant allele restored pollen germination frequency to wild-type levels. However, quantitative assessments of pollen grain diameter, nuclei count, viability, and hydration showed no differences between wild-type and mutant, arguing that *myo9* is dispensable for pollen grain development. Rather, we conclude that *myo9* is required specifically for initiation of pollen tube growth following hydration.

Gene / Gene Models described: *myo9*, *ZmXI-11*; GRMZM2G435294, Zm00001d044819

Funding acknowledgement: National Science Foundation (NSF), OSU College of Agricultural Sciences

P178 

## A novel maize glycosyltransferase is required for carbon export from source tissues

(submitted by Tyler McCubbin <[tm284@mail.missouri.edu](mailto:tm284@mail.missouri.edu)>)

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As autotrophs, plants must transport the carbon that is fixed in the photosynthetic source tissues, such as leaves, to the non-photosynthetic sink tissues, such as roots or reproductive tissues. This process, known as carbohydrate partitioning, is essential for plant growth and survival, and requires coordinated action by many enzymes and transporters.

Here we describe a recessive mutant with carbohydrate partitioning defects, including reduced growth, reproductive defects, and carbohydrate hyperaccumulation in leaves. We identified three alleles of the causal gene, which were all single amino acid mutations that mapped to a putative glycosyl transferase on chromosome 9. Little is known about the biochemical function of the predicted protein, which we show to be a Golgi resident. Preliminary analyses of cell wall chemistry identified altered carbohydrate linkages, which are characteristic of changes in arabinogalactan glycosylation and arabinoxylan branching, suggesting that Cpd7 may function in cell wall biosynthesis or remodeling.

To further characterize these mutants, we conducted a histological analysis that revealed ectopic lignin deposits in the phloem of mature leaves. These deposits occurred in a developmentally progressive pattern. We hypothesize that these lignin deposits perturb long-distance transport of sucrose, interfering with source-to-sink carbon transport. Radiotracer experiments revealed decreased basipetal transport of sucrose in source leaves, suggesting that perturbed long-distance sugar transport underlies the cpd7 phenotype. Ongoing efforts will further elucidate of the link between cell wall composition and carbohydrate partitioning.

Gene / Gene Models described: ; Grmzm2g161293

Funding acknowledgement: National Science Foundation (NSF)



P179 

## **A promising new approach: Characterization of the maize hypersensitive response using fluorescence tagged transgenic lines.**

(submitted by BongSuk Kim <[kim1@purdue.edu](mailto:kim1@purdue.edu)>)

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The hypersensitive response (HR) is a well-known mechanism of programmed cell death against biotic stress in plants. Despite numerous studies on this subject, our understanding of how cell death underlying HR is initiated and spread throughout the cell remains fuzzy. Some studies suggest chloroplasts as the organelle responsible for HR initiation while others claim it is the vacuole or the plasma membrane that provide the initiating site for cell death. However, there is lack of direct evidence supporting all those claims. To address this issue, we have obtained 50 different maize lines in which every organelle or compartment of a cell has been marked with a fluorescent protein (FP). After backcrossing these lines to B73 over at least 3 generations, they have been crossed with Rp1-D21, an autoimmune mutant in which the initiation of spontaneous HR lesions can be controlled by temperature. Lesions only form when the plants are grown at temperatures below 28 degree C. This allowed us to initiate HR lesions at will and monitor by confocal imaging of the nature of cellular collapse. Results thus far show that destruction of the chloroplast and nucleus happens first while the cell wall and plasma membrane remain intact at the earlier times. We are also working on some cellular and biochemical assays to go with confocal imaging to pinpoint the initiation and spread of cell death during HR.

Funding acknowledgement: National Science Foundation (NSF)

P180 

## **Analysis of an inflorescence mutant in foxtail millet (*Setaria italica*)**

(submitted by Molly Haddox <[molly.haddox@okstate.edu](mailto:molly.haddox@okstate.edu)>)

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Inflorescence development in grasses is highly diverse and serves as a critical diagnostic character for specific clades. Within the subfamily Panicoideae, inflorescences are typified by a spikelet morphology consisting of two florets, each being subtended by a lemma, all of which are encased within paired bracts (glumes). Although the genetic pathways for floral development are best understood in *Zea mays*, a unique homeotic mutant was identified in the emergent model for the panicoid grasses, *Setaria italica*, that can help to expand our knowledge of floral development within the panicoid grasses more broadly. The chia mutant was identified in N-Nitroso-N-methylurea mutant screens of *S. italica acc. Yugu1*, due to the presence of its distinct leafy panicle phenotype. Initial observations suggest that this phenotype results from abnormalities in the developmental transition and determinacy of spikelets. In order to identify genetic regions associated with this phenotype, a bulk segregant analysis (BSA) was performed on eight *chia* mutants. The BSA results revealed high frequency recombination blocks associated with this phenotype. In addition, mutant spikelets were analyzed in order to characterize their morphology more fully. The BSA results, along with the morphological data, can help to enlarge on our knowledge of panicoid development.

Funding acknowledgement: National Science Foundation (NSF)



**P181**

**Analysis of *stunter2* and *stunter3*, maize maternal effect mutants with reduced kernel size**

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Regulation of growth and development of seeds in plants is largely controlled by the haploid female gametophyte through gene expression following meiosis. *stunter2* (*stt2*) and *stunter3* (*stt3*) are novel maize mutants that disrupt proper development of the female gametophyte, which ultimately affects seed development post fertilization. These two mutants phenocopy *stunter1* (*stt1*), a previously characterized maize mutant with viable but reduced embryos and endosperms and small female gametophytes (Phillips and Evans, 2011). *stt2* and *stt3* embryo sacs are smaller, with smaller central cells and fewer antipodal cells than wild type. Additionally, both mutants exhibit reduced transmission through the male gametophyte. Like *stt1*, *stt2* and *stt3* pollen grains are smaller, but it is unclear if the mutations affect pollen tube germination. Post-fertilization, both embryo and endosperm development is delayed, with *stt2* and *stt3* exhibiting disruptions in the development of the basal endosperm transfer layer, which controls nutrient transport to the developing seed. Whereas *stt2* may be allelic to *stt1*, *stt3* is unlinked and represents a unique lesion. These mutants will help elucidate mechanisms for maternal control of seed development in maize.

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

**P182**

**Analyzing the role of boron in meristem maintenance**

(submitted by Zoe Darnell <[zedkwv@mail.missouri.edu](mailto:zedkwv@mail.missouri.edu)>)

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Boron is an essential micronutrient with a vital role in stabilizing the cell wall, though evidence suggests that it also plays other functions within the plant cell. The *tls1* gene encodes a boron importer required for vegetative and inflorescence development in maize with the first defects being observed in meristems (a group of stem cells). Our lab showed that both the shoot apical (SAM) and inflorescence meristems are much smaller in the boron deficient *tls1* mutant compared to normal ones, indicating that *tls1* has defects in maintaining the proper number of stem cells. This results in *tls1* lacking the tassel (male inflorescence) and the ear (female inflorescence). To assess whether the defects seen in the SAM are progressive, we analyzed SAM size in mature embryos with histology and observed that meristem size is altered in mature *tls1* embryos compared to normal embryos. The size of a meristem is regulated by the CLAVATA-WUSCHEL (CLV-WUS) feedback loop. To better understand the observed meristem maintenance defects in *tls1*, we analyzed double mutants of *tls1* with *thick tassel dwarf1* (*td1*), which encodes a member of the CLV-WUS pathway. The *td1* mutants are typically short with thick tassels and fasciated ears, due to an overproliferation of stem cells. Our results show that both the tassel and the ear phenotypes of *tls1* can be partially rescued by *td1*, which indicates a functional involvement of boron during meristem maintenance, and also points to additional cellular functions of boron beyond the cell wall.

Funding acknowledgement: United States Department of Agriculture (USDA)

P183 

## Auxin signaling and grass development

(submitted by Annis Richardson <[annisrichardson@berkeley.edu](mailto:annisrichardson@berkeley.edu)>)

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Plant architecture and organ shape are intricately linked with plant productivity. The phytohormone auxin has been identified as a core player in patterning both architecture and organ shape. The diverse roles of auxin are facilitated by a large signaling network, involving multiple transcriptional regulators, Auxin Response Factors (ARFs), and their interacting partners AUX/IAAs. Although auxin signaling has been studied in eudicot models, far less is known in grass models. In maize there are 33 ARFs and 38 AUX/IAAs. To date, few mutants in maize have been reported in AUX/IAAs and none are reported in ARFs, suggesting that there is a high level of functional redundancy. Here we present two mutants, *Hoja loca* and *Truffula*, which have severe defects in leaf development and plant architecture. *Hoja loca* is a semi-dominant mutant, which has a mutation in the degron motif of the AUX/IAA protein IAA38. The constitutive activity of IAA38 leads to node skipping and the development of midrib-less and tube leaves. Patterning of organ primordia is normal, but the incipient primordium fails to grow, suggesting that IAA38 is involved in decoding the auxin signal for growth promotion in the meristem. This role does not appear to be conserved in other outgrowths like the ligule which is normal in mutant leaves. Conversely, *Truffula* (*Trf*) mutants develop many extra leaves with aberrant phyllotaxy, generating plants with an architecture reminiscent of the Dr Seuss truffula trees. *Trf* is a dominant mutant which has been mapped to a region on chromosome 10. We have identified a *Trf* candidate mutation in ARF28 in the dimerization domain and we are currently testing the effects of the mutation on protein structure and dimerization ability. Through these studies we hope to shed light on AUX/IAA and ARF signaling in maize and the regulation of plant architecture and organ shape.

Gene / Gene Models described: ; GRMZM2G035465, GRMZM2G006042

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

P184 

## Branching out, exploring the genetic landscape of branching in *Setaria viridis* (green foxtail) with the *cushion plant* mutant background

(submitted by John Hodge <[jghodge@okstate.edu](mailto:jghodge@okstate.edu)>)

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*Setaria viridis* is a relatively unexplored model system for molecular genetics in panicoid grasses, with the advantages of rapid generation time, short stature, and small sequenced genome. We used this system to analyze a mutant with a severe branching phenotype that appeared insensitive to differing environmental conditions. The *cp* mutant, named for its *cushion plant* habit, exhibits a constitutive branching phenotype, changes in culm height, and leaf size along the main culm. An F2 population was generated from two independent backcrosses of a *cp* mutant to the A10.1 wild-type background, was used to perform MutMap bulked segregant analysis in order to map the causative region. In addition, the differences between mutant and wildtype development were characterized by function value trait (FVT) analysis for culm height, leaf number, branch number, and leaf area. Branch number FVT's were then averaged by wild-type and mutant morphogroups in order to capture the developmental timepoint in which segregation of genotypes first occurs. The point in time where average FVT's segregate was then used to identify an informative timepoint for targeted gene expression analyses of axillary buds. Genetic markers for meristem activity (*KNOTTED1*) and axillary bud dormancy (*TEOSINTE BRANCHED1*, *DORMANCY1*, and *SQUAMOSA PROMOTER-LIKE14*) were assayed between mutant and wild-type lines prior and leading up to the point of averaged FVT segregation between genotypes. This gene expression analysis performed in the context of our FVT's allowed the *cp* mutation to be characterized in *S. viridis* within the context of known developmental network of branching regulators in other grass species.

Funding acknowledgement: National Science Foundation (NSF)

P185 

### **Characterization and mapping of the *Suppressor of sessile spikelet3 (Sos3)* mutant which functions in meristem maintenance in maize**

(submitted by Amanda Blythe <[amb4x2@mail.missouri.edu](mailto:amb4x2@mail.missouri.edu)>)

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Stem cells play a pivotal role in providing the cells necessary for proper growth and development. In plants, stem cells are housed in a microenvironment termed the meristem. Within the meristem, stem cells respond to a variety of regulatory signals aiding in meristem maintenance. One of the most widely studied meristem maintenance pathways is the *CLAVATA-WUSCHEL (CLV-WUS)* pathway, which regulates the expression of the transcription factor WUS to maintain the meristem size. In order to study meristem maintenance within maize, the *Suppressor of sessile spikelet3 (Sos3)* mutant is being analyzed. *Sos3* is a semidominant mutant that produces single spikelets (short branches that produce the florets in maize) instead of the typical paired spikelets, causing defects in both the male (tassel) and female (ear) inflorescences. SEM and histology analyses show evidence of single spikelet production in both the tassel and ear of *Sos3* mutants. Additionally, there is evidence that *Sos3* mutants have smaller inflorescence meristems compared to wildtype, indicating a disruption in meristem maintenance. In order to further test these results, double mutant analyses with other meristem mutants, such as *td1* and *fea2* are currently underway. Overall, these results indicate that the *sos3* gene functions in meristem maintenance and potentially functions in the *CLV-WUS* pathway. Sequencing information is being analyzed to determine the location and function of the *sos3* gene. Understanding the precise function of the *sos3* gene could lead to increased knowledge of paired spikelet development within grass species, as well as a better understanding of the mechanisms regulating meristem maintenance in maize. In both cases, applying this knowledge could lead to increased yields in important cereal crops, thus having major agricultural impacts.

Funding acknowledgement: National Science Foundation (NSF)

P186 

### **Characterization and sequencing of maize mutant *Suppressor of sessile spikelet 3 (Sos3)***

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Maize, wheat, and rice are three of the most important crops in the world, providing food for billions of people, which makes research in these crops essential. A key difference between these plants is that corn produces its fruit (the kernels) in paired rows, while wheat and rice produce single rows of their fruit, resulting in corn yield being doubled. In maize, a developmental pathway consisting of multiple meristems gives rise to the paired rows of kernels on the ears. However the *Sos3* mutant in maize produces single rows of kernels and looks more akin to wheat and rice. My interest in the *Sos3* mutant is that if we can identify the mutant gene responsible for the reduction in the kernel row number on the ears of maize, then we may be able to transfer the non-mutated version of the gene to wheat and rice which could theoretically double the yield in these crops in the future. My project focused on finding and characterizing the mutant gene causing *Sos3*. Next generation sequencing techniques were used to identify the genes that were most likely to be contributing to the *Sos3* mutant phenotype. The next goal of the project was to attempt to discover which biochemical pathway the *Sos3* gene functioned in. We accomplished this goal by crossing *Sos3* with *td1* in the field and observing the resulting double mutant phenotype. This project will hopefully provide better understanding and insight into the development and evolution of the reproductive structures in maize.

Funding acknowledgement: National Science Foundation (NSF)

P187

**Characterization of increased *Pantoea stewartii* resistance in maize *pan1* mutants**  
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*Pantoea stewartii* is a gram negative bacterium that is the etiological agent of Stewart's wilt, the most agronomically significant bacterial disease of maize and sweet corn in the Midwest and Northeast of the USA. It is a hemibiotrophic vascular pathogen that is vectored by the corn flea beetle, which introduces the bacterium into both the intercellular spaces of the leaves, where it causes water-soaked lesions (WSL), and the vasculature. *P. stewartii* preferentially colonizes the xylem, leading to systemic spread throughout the plant and characteristic wilting symptoms. PAN1 is an enzymatically inactive leucine-rich repeat receptor-like kinase originally described as a regulator of stomata development. We discovered that maize *pan1* null mutants, which have stomatal defects in juvenile leaves but display normal growth and morphology, show dramatically increased resistance to *P. stewartii*. We found that *pan1* mutants are more resistant to Stewart's Wilt disease by impairing *P. stewartii* xylem colonization and spread, but not its ability to cause WSL. Our results suggest that an enhanced vascular defense response that involves the accumulation of host-derived material in xylem vessels to prevent pathogen spread can be a key factor that contributes to *P. stewartii* resistance in *pan1* mutants.

Funding acknowledgement: United States Department of Agriculture (USDA)

P188

**Characterization of *ramosa suppressor locus\*12.2995*, a likely novel allele of *opaque1* that regulates plant architecture in maize**  
(submitted by Brian Zebosi <[bzebosi@iastate.edu](mailto:bzebosi@iastate.edu)>)

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Ideal plant architecture optimizes canopy structure and increases grain yield in maize. However, its underlying genetic mechanisms remain poorly characterized. We recently identified and characterized a recessive, EMS-induced maize mutant, *ramosa suppressor locus\*2995.12* (*rsl\*12.2995*) with aberrant plant architecture. *rsl\*12.2995* mutant is semi-dwarf with compressed internodes, reduced tassel branch number and opaque kernels. It also has displays reduced leaf size, extremely narrower leaf midrib, reduced auricle and ectopic ligule tissue displaced into the midrib. We also investigated the genetic interaction between *rsl\*12.2995* and *ramosa* mutants, which show increased branch number in the tassel and branched ears. *rsl\*12.2995* partially suppresses *ra1* and *ra2* tassel phenotypes. Using map-based cloning and whole genome sequencing, we localized *rsl\*12.2995* to a small region on Chr4 containing a missense mutation in a gene encoding a Myosin XI motor protein, the same gene as *opaque1* (*o1*) in maize. Allelism tests are underway. Based on these results, we propose that *rsl\*12.2995*, a likely novel allele of *o1*, plays a role in leaf patterning, inflorescence development and overall plant architecture.

Gene / Gene Models described: *o1 - opaque endosperm1*; GRMZM2G449909

Funding acknowledgement: National Science Foundation (NSF)

P189

## Characterizing the maize *CLAVATA3/EMBRYO SURROUNDING REGION* (*CLE*) genes function by CRISPR/Cas9 genomic editing technology

(submitted by Lei Liu <[lliu@cschl.edu](mailto:lliu@cschl.edu)>)

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The development of shoot meristems is maintained by CLAVATA–WUSCHEL feedback signaling between the stem cell zone at the tip of the meristem and the underlying organizing center. The CLE (*CLAVATA3/ EMBRYO SURROUNDING REGION*) genes encode a major group of secreted peptides that function as signals in cell-cell communication, cell proliferation and differentiation. In Arabidopsis, CLV3 is secreted from stem cells at the tip of the meristem and perceived by leucine-rich-repeat (LRR) receptors, such as CLV1, to repress WUS expression. However, most of the CLEs in maize have not been studied, and only *ZmCLE7*, thought to be the CLV3 ortholog, and *ZmFON2-LIKE CLE PEPTIDE1* (*FCP1*), were proposed to bind to two separate FASCIATED EAR2 (*FEA2*) receptor complexes, with distinct downstream signals passing through CORYNE (*ZmCRN*) or the alpha subunit of maize heterotrimeric G protein COMPACT PLANT2 (*CT2*) separately; and *ZmFCP1* can also be perceived by the FASCIATED EAR3 (*FEA3*) receptor.

To survey the function of maize CLEs, especially their function on meristem development, we collected all the predicted maize CLE sequences, which suggested there are 49 predicted CLEs in maize. Then we integrated the expression data of multiple maize tissues and selected 31 genes with higher expression in the inflorescences to create mutations by the genomic editing technology CRISPR/Cas9. Up to now, frameshift mutations have been induced in 27 of the 31 CLEs, and cle mutants from the same subgroup are being crossed together to evaluate their functional redundancy.

We are also interested in the idea of creating weak alleles to improve agronomic traits such as kernel row number. As *Zmfcp1* and *Zmcle7* single mutants have a fasciated ear phenotype, we CRISPRed their promoters to create weak alleles to enhance maize yield traits. Preliminary phenotyping of *ZmCLE7* promoter weak alleles showed a significantly enlarged but non-fasciated ear, indicating potential usage of these favorable alleles in maize breeding.

Gene / Gene Models described: *ZmCLE7*, *ZmFCP1*, *ZmCRN*, *FEA2*, *FEA3*, *CT2*; GRMZM2G372364, GRMZM2G165836, GRMZM2G032132, GRMZM2G104925, GRMZM2G166524, GRMZM2G064732  
Funding acknowledgement: National Science Foundation (NSF)

P190

## Cloning and characterization of classical maize mutant, *Polytypic1*

(submitted by Anastasia Amoigroulou <[amoigroulou12@students.ecu.edu](mailto:amoigroulou12@students.ecu.edu)>)

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*Polytypic1* (*Pt1*) is a semi-dominant mutant that affects inflorescence development. To understand how *Pt1* functions in development, we examined the phenotype of *Pt1* mutants in multiple inbred backgrounds. *Pt1* defects are restricted to the inflorescence, but the severity of the phenotype is background dependent. In B73, *Pt1/+* mutants have severe floral defects; floral meristems initiate ectopic floral organs and *Pt1/+* ears are female sterile. *Pt1/+* tassels have similar defects as the ear, but are less severe. *Pt1/+* tassels contain fewer spikelets, and florets often produce extra floral organs. Because *Pt1/+* ears are sterile in B73, we could not examine the *Pt1* homozygous phenotype. In A619, however, the *Pt1* phenotype is mild and *Pt1/+* are female fertile. In A619, both ears and tassels from *Pt1* homozygotes are pin-like with severe reduction in lateral primordia, indicating *Pt1* has broader roles in inflorescence development. To identify the gene responsible for the *Pt1* mutant phenotype, we pursued a positional cloning approach coupled with RNA-seq. We mapped *Pt1* to a 6.8cM interval (~5Mbp) on chromosome 6 and performed RNA-seq to identify RNAs with changes in expression levels or sequence. The *Pt1*-containing interval contains 111 genes that are expressed in ear primordia, nine of which are differentially expressed (FC>2, padj< 0.05). However, none of these genes are striking candidates. We are currently analyzing our data to identify RNAs with sequence changes that could be responsible for the *Pt1* mutant phenotype. We are also analyzing our RNA-seq data to determine how *Pt1* affects gene expression. Notably, 13 MADs-box transcription factors, which are known floral regulators, are dramatically downregulated in *Pt1* mutants. The characterization of *Pt1* mutants will give insight into the mechanisms that underlie normal inflorescence development.

Funding acknowledgement: National Science Foundation (NSF)

P191

## **Confocal analysis of expression patterns of DR5 and PIN1 in normal developing maize embryos.**

(submitted by Dale Brunelle <[dale.brunelle@und.edu](mailto:dale.brunelle@und.edu)>)

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The developing maize embryo passes through the proembryo, transition, coleoptilar and stage 1 (first leaf primordium) morphogenetic stages followed by the iterative formation of additional leaf primordia during stages 2 through 6 according to Abbe and Stein (1954). Using ethyl methanesulfonate (EMS) we have previously produced lethal embryo specific (*emb*) mutants that have no obvious effects on endosperm development except for some reduction in kernel size in some cases. The embryo phenotypes of 34 mutations were examined by dissection of the mature embryos and reported (Brunelle, Clark and Sheridan, 2017). All were retarded in development and morphologically abnormal. Thirty-two of the embryos were blocked in development in the late proembryo stage to stage 1. The abundance of EMS-induced mutations blocked early in embryo development suggest an abundance of genes acting during this period to regulate the changing patterns of signaling molecules that underlie the cellular changes occurring during development. In order to further understand embryo development, evaluation of the expression patterns for 10 fluorescent protein constructs in normal developing embryos has begun. Two of the constructs used to report auxin expression are presented here: DR5 and PINFORMED1 (PIN1). DR5 is synthetic auxin response element that reports on expression seen in auxin inducible tissues. Expression of DR5 has been observed as early as the transition stage and continues to stage 1. PIN1 is an auxin efflux transporter and is localized in the plasma membrane. PIN1 expression is seen at the proembryo stage to stage 1. The evaluation of expression patterns in normal embryos for DR5, PIN1 and the other eight proteins will be used as a baseline to compare to expression of the same proteins in the *emb* mutants. The work performed here is foundational to understanding the genes involved in embryogenesis and will aid in developing a systems biology for embryogenesis.

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

P192

## **Corn RHS2, a tassel-specific siRNA-mediated glyphosate inducible male sterility system for commercial production of hybrid seed in maize**

(submitted by Balasulojini Karunanandaa <[bala.karunanandaa@bayer.com](mailto:bala.karunanandaa@bayer.com)>)

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Hybrid crops produce higher yields than their inbred parents due to heterosis. For high purity of hybrid seeds, it is critical to eliminate self-pollination. Roundup® Hybridization System (RHS) utilizes glyphosate to induce male sterility, which eliminates the need for manual or mechanical detasseling for commercial hybrid seed production. The first-generation RHS (RHS1) is based on low expression of a glyphosate-insensitive 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) in pollen. The second-generation RHS (RHS2) technology is built on RNA interference (RNAi) combined with CP4 EPSPS. It utilizes maize endogenous male tissue-specific small interfering RNAs (mts-siRNAs) to trigger cleavage of the CP4 EPSPS mRNA specifically in tassels, resulting in glyphosate-sensitive male cells. Male sterility is then induced by glyphosate application at the stages critical for pollen development, and the male-sterile plants are used as the female parent to produce hybrid seed. The endogenous mts-siRNAs are conserved across maize germplasm, and the inducible male sterility was replicated in representative germplasm through introgression. This technology combines the relative simplicity and convenience of a systemic herbicide spray methodology to create an inducible male sterility system for industrial hybrid seed production.

Funding acknowledgement: Bayer Crop Science



P193 

### **Defining the developmental program leading to meiosis with single-cell RNA-seq**

(submitted by Brad Nelms <[bnelms.research@gmail.com](mailto:bnelms.research@gmail.com)>)

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The entry into meiosis is a complex process characterized by increasing levels of meiotic specialization. We used single-cell RNA-sequencing to reconstruct the developmental program into meiosis in maize male flowers. We observed a smooth continuum of expression stages leading up to meiosis, followed by a sharp reorganization of the transcriptome in early meiotic prophase – involving a 2-fold or greater change in 26.7% of expressed genes. Changes in cell physiology accompanied the nuclear events of meiosis, including a decrease in protein translation capacity and increase in membrane-bound organelles. Analysis of cell cycle-correlated gene expression indicates all pre-germinal cells proliferate, eliminating a stem-cell model. Our results uncover a multi-step pathway into meiosis and highlight the power of single cell RNA-seq to define developmental transitions.

Funding acknowledgement: National Science Foundation (NSF)

P194 

### **Development of regenerable maize ExPVP-BC lines for genome engineering and editing applications**

(submitted by Frank McFarland <[fmcfarland@wisc.edu](mailto:fmcfarland@wisc.edu)>)

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Recent advances in the creation of ‘genotype-independent’ transformation systems in maize have been made, however those systems retain some logistical hurdles, including utilization of a complicated, proprietary vector system, genotype-dependent differential plant regeneration rates, and IP restrictions that can impede use by public researchers or transformation services. As a result, many labs still rely upon publicly available systems targeting a limited number of regenerable maize genotypes such as B104, Hi II AxB, or A188. In an effort to expand the array of elite, publicly targetable genotypes and to study the genetic mechanisms underlying somatic embryogenesis and plant regeneration, we developed 65 BC 2 lines from crossing the transformable line A188 to 13 different non-regenerable ExPVP inbred lines. ExPVP lines are older elite, commercial inbreds that are publicly available because their patents have expired. BC 2 plants were grown and crossed to their recurrent parents to produce BC 3 progeny. At 10-12 days after pollination, BC 3 immature embryos were isolated and plated onto callus induction medium. Seventy-three ears were evaluated. Twenty-five embryos from each ear were plated and maintained through several rounds of sub-culturing. Embryos that produced friable, embryogenic callus were identified and regenerated to produce R 0 plants. Seven regenerable, independently-derived BC 3 sources were identified across four ExPVP backgrounds: LH51, LH195, LH198 and DK78004. R 0 plants were self-pollinated and backcrossed onto the recurrent parent to produce BC 4 ears. Multiple rounds of selection and purification will be necessary to produce inbred lines that are homozygous for the A188 introgressions which confer high culture response. Previously, we identified a 3Mb region on the long arm of chromosome 3 significantly associated with A188-derived high tissue culture response. The regenerable BC lines identified here should help in further mapping of the genes conferring tissue culture response and in understanding the genetic basis for somatic embryogenesis in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)



P195

## Differential gene expression in the upper and lower floret of maize

(submitted by Hailong Yang <[yanghal6@students.ecu.edu](mailto:yanghal6@students.ecu.edu)>)

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Flowers are essential for plant reproduction and also produce seeds and fruits that are consumed as food. In maize, male and female flowers are borne on separate inflorescences; the tassel produces male flowers and the ear produces female flowers. Grass flowers (called florets) are contained in spikelets. Maize spikelets initiate two floral meristems that give rise to the upper floret and lower floret. In the tassel, both florets fully develop, resulting in mature spikelets with two male florets. In the ear, the lower floret aborts, resulting in mature spikelets with a single female floret. Maize florets contain the grass-specific organs, lemma, palea, and lodicules, in addition to stamens and carpels. Sex determination occurs through pistil abortion in the tassel and stamen arrest in the ear.

To understand the gene regulatory networks that function in floral development, we used laser capture microdissection coupled with RNA-seq to identify genes specifically expressed in the upper and lower floral meristems. Approximately 600 genes are differentially expressed (DE) between the upper and lower florets ( $FC \geq 2$ ;  $q < 0.05$ ) and are enriched for genes involved in transcriptional regulation, development and hormone metabolism. We used RNA in situ hybridization to examine the expression of five DE genes, all of which have distinct expression patterns in the floral meristem; three of these genes appear to be differentially expressed between the upper and lower floral meristems. These analyses have the potential to uncover new genes and regulatory networks that function in maize floral development.

Funding acknowledgement: National Science Foundation (NSF)

P196

## Dissection of a genetic network that cross-talks with brassinosteroids to modulate axillary meristem fate and inflorescence architecture

(submitted by Jiani Yang <[jyang@danforthcenter.org](mailto:jyang@danforthcenter.org)>)

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Inflorescence architecture is an important agronomic trait that influences yield potential. Inflorescences are shaped by the position and fates of axillary meristems (AMs). In grasses, AMs eventually terminate in spikelets, which produce flowers and grain. In *Setaria spp.*, AMs differentiate into either a spikelet or a sterile “bristle”, and these structures appear to be paired. We previously characterized the *bristleless1* (*bsl1*) mutant in *Setaria viridis*, which produced few to no bristles because bristles were homeotically converted to spikelets. The *bsl1* gene encodes a rate-limiting enzyme in brassinosteroid (BR) biosynthesis. While this phenotype provides an avenue for understanding the genetic basis for spikelet differentiation, our knowledge of the underlying mechanisms is very limited. To gain further insight, we screened for additional mutants with defective bristle and/or spikelet production in a mutagenized population of *Setaria* and found *spikeletless* (*spkl*), which exhibited the opposite phenotype, producing only a few spikelets. SEM analyses showed that *spkl* mutant spikelets were homeotically converted to bristles, similar to the *bsl1* mutant phenotype, but directly opposite. We further analyzed genetic interactions between the mutants and tested the effects of BR inhibitor treatments on mutants to establish a genetic hierarchy, which indicated *bsl1* is epistatic to *spkl*.

To study the comparative functions of *bsl1* in spikelet differentiation, we evaluated the spatiotemporal expression of maize ortholog of *bsl1*, *Zmdwarf11* (*d11*), by *in situ* hybridization, which revealed its expression in analogous domains (at the base of spikelet pair and spikelet meristems) in early inflorescence development. We also characterized a uniform-Mu mutant allele of *Zmd11* which showed similar phenotypes to *bsl1* mutants, such as semi-dwarf stature and short pedicels along tassel branches. These results suggest some conserved function of this BR biosynthesis gene in *Setaria* and maize development. Moving forward, our analyses of *bsl1* and *spkl* in *Setaria* and their orthologs in maize will provide insight into the mechanisms that integrate BRs in modulating meristem fate and diverse inflorescence morphologies in grasses.

Funding acknowledgement: National Science Foundation (NSF)

P197 

## **Diversity in cuticle composition: A survey across maize development for two inbreds and among silks of the Wisconsin Diversity Panel**

(submitted by Travis Hattery <[thattery@iastate.edu](mailto:thattery@iastate.edu)>)

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The plant cuticle is synthesized by the epidermis and consists of a cutin matrix infused with and coated by non-polar and amphipathic cuticular lipids that vary in composition depending on organ and developmental stage. This cuticular layer accumulates on aerial portions of plants and provides a protective, hydrophobic barrier against both biotic and abiotic stresses. The genetic networks that organize the synthesis, transport, and localization of cuticular components are not fully understood. To dissect these genetic networks, we have profiled the silk cuticular lipid metabolome from 500 genetically diverse maize inbred lines contained within the Wisconsin Diversity (WiDiv) Panel which flower within a two-week time window. Cuticular lipids were extracted from silks that had emerged from encasing husk leaves and were profiled via gas chromatography and flame ionization detection. A high-throughput metabolomics analysis pipeline has revealed a set of 47 unique lipid metabolites. Herein, we present a summary of metabolome compositions from the WiDiv panel grown in two locations (Minnesota and Iowa) as well as in two growing seasons (Summers 2016 and 2017), demonstrating over 17-fold variation in surface lipid accumulation. In the future, genome wide association studies will be conducted to understand the underlying genetic networks controlling these metabolites and how this genetic network interacts with environmental (GxE) perturbations. To further explore the diversity in cuticle composition, we have profiled both the cuticular lipid fraction as well as the cutin monomers from nine developmental stages from inbreds B73 and B104, including leaves (V11), tassels, immature ears, and whole kernels. We have identified a set of ~130 unique cuticular lipid metabolites that exhibit up to a 140-fold difference in accumulation across tissues. In the future, this cuticular lipid atlas will be combined with transcriptome and proteome data to become a powerful tool for future genome survey studies.

Funding acknowledgement: National Science Foundation (NSF)

P198 

## Diversity of abscission zone development and underlying transcriptomic regulation in grasses

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Seed shattering is a process in which seeds separate from the parent plant, which is essential for seed dispersal. In agriculture, reduced shattering or non-shattering has been selected in cereal crops to facilitate seed harvesting. Seeds separate from their stalk at one or a few specialized cell layers called the abscission zone (AZ). In the grass family, the AZ may form below the fruit, below the flower in the rachilla, below sets of flowers in the pedicel or rachis, or even below the entire inflorescence, raising the question of how much of the underlying genetics is shared among different species. This study investigates three grass species, a shattering weedy rice (*Oryza sativa*) and *Brachypodium distachyon* with AZs in the rachilla, and *Setaria viridis* with an AZ in the pedicel, to compare AZ development at anatomical and molecular levels. We find that AZs of rice and *Brachypodium* are both composed of one or two layers of small cells different from the adjacent cells, while the AZ of *Setaria* is not histologically distinct. Transmission electron microscopy shows that the AZ of rice is thin-walled and lacks lignin deposition, while that of *Brachypodium* is thick-walled with lignin, suggesting different AZ anatomy even with the same AZ position. RNA-seq analysis comparing the AZ and regions immediately above and below it in all three species found that the overlap of genes enriched in the AZ is small in all pairwise species comparisons (see companion poster by Hu et al.). In situ hybridization of previously identified shattering genes also showed diverse temporal and spatial expression patterns in different species. Together, these results suggest that although the AZs serve the same ecological function, their developmental program and transcriptional regulation in different species may vary dramatically, resulting in morphological diversity.

Funding acknowledgement: National Science Foundation (NSF)

P199

## Does spikelet/kernel abortion follow a modified leaf growth and senescence program?

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Improving grain yield is a major objective of crop breeding, and one promising avenue for maximizing yield is through enhanced spikelet/kernel survival that increases grain number. Grain crops like barley and maize form indeterminate inflorescence meristems which develop spikelets in acropetal succession. The grain-bearing structures of these crops exhibit spikelet abortion following a basipetal gradient even in optimal conditions. During unfavorable conditions, spikelet abortion becomes more severe and sharply reduces the yield. Though several hypotheses, such as competition for assimilates, the position of the spikelets and pollination time gap, were proposed for the cause of spikelet abortion, the mechanism of spikelet abortion is still elusive. From our comprehensive study on barley spikelet development and growth, we found that during the spikelet development phase, the inflorescence meristem and initiated spikelets are in an active state of mitosis. However, before the end of the spikelet development phase, we already detected mitotic arrest in the cells surrounding the inflorescence meristem. In later stages of development, we clearly observed that the mitotic arrest starts moving basipetally from the inflorescence meristem to the developed spikelets and is followed by the abortion of spikelets in the same basipetal pattern. Since Goethe's path-breaking finding in 1790, we already knew that floral parts (inflorescence) are nothing else but modified leaves. By applying Goethe's conception to our observed pattern of spikelet abortion, we hypothesize that it resembles the movement of the cell cycle arrest front followed by programmed cell death in the leaf growth and senescence program. This intriguing idea, however, certainly needs a further thorough testing in the future but may open up new avenues in understanding spikelet abortion in cereals and thus might help in improving the grain number and yield.

Funding acknowledgement: European Research Council (ERC)

P200 

## **Endogenous jasmonic acid levels are high in juvenile maize leaves**

(submitted by Krista Osadchuk <[krista-osadchuk@uiowa.edu](mailto:krista-osadchuk@uiowa.edu)>)

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As the shoot of an angiosperm develops it undergoes the transition from the juvenile to the adult vegetative phase, ultimately leading to flowering. In maize, these phases differ in both gene expression patterns and leaf characteristics. Previous research demonstrated that the juvenile-to-adult vegetative phase transition is mediated by leaf derived signals. Leaf primordia formed in the juvenile phase highly express genes that relieve oxidative stress and facilitate jasmonic acid (JA) signaling. Exogenous application of JA extends the juvenile phase and increases the expression of miR156, the principle regulator of the juvenile phase. Here we tested whether JA is the leaf-derived cue that promotes the juvenile phase. We measured JA using LC-MS in successive stages of leaf one development and leading up to phase change in normal maize and phase change mutants. We concurrently measured gibberellic acid (GA), a plant hormone that promotes germination and vegetative phase change. To investigate when gene regulation by miR156 begins in the context of JA and GA signaling, we analyzed gene expression patterns through germination and leading up to phase change. While continued JA treatments could not extend the juvenile phase indefinitely, endogenous levels of JA increase from germination through leaf one differentiation, declining in later formed leaves as the shoot approaches the adult vegetative phase. In contrast, levels of GA are low in leaf one after germination and increase as the shoot transitions to the adult phase. Our hypothesis that the stresses of germination and seedling establishment initiate the juvenile phase through JA signaling is supported by gene expression patterns in the embryo during imbibition.

Funding acknowledgement: National Science Foundation (NSF)

P201

## **Genetic characterization of Korean waxy maize landraces by RNA sequencing**

(submitted by Gbum Yi <[gibumyi@gmail.com](mailto:gibumyi@gmail.com)>)

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Maize (*Zea mays* L.) is the second-most produced crop in the Korean peninsula after rice. Maize has been continuously cultivated in Korea since the middle of the 16th century, when it was originally introduced from China. Even with such extensive cultivation history, the diversity and properties of Korean landraces have not been investigated at the DNA sequence level. We collected twelve landraces with various flowering times from National Agrobiodiversity Center in Korea and performed RNA sequencing in the early vegetative stage. The transcriptomes of twelve Korean landraces have been analyzed for their genetic variations in coding sequence and genetic relationships to HapMap2 population. The Korean landraces showed specific genetic characteristics and were clustered together with a Chinese inbred line. Flowering-time related gene profiles suggested that there were at least two causes of the variation of flowering time within Korean landraces and showed significant positive and negative correlations among genes inferring possible flowering time regulation mechanisms unrevealed in maize.

Funding acknowledgement: Rural Development Administration, Republic of Korea

P202

## Genomic and phenotypic drivers of agronomic gain via an EMS-treated sorghum population

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Sorghum (*Sorghum bicolor* (L.) Moench) has become a viable C4 crop and agronomic model for Poaceae research. Improved cost and depth of whole-genome sequencing has permitted the rapid mapping of useful mutants cultivated from phenotypic screens. An EMS-mutagenized population of BTx623 seeds was created to advance trait prediction and characterization; this germplasm population contains a high genome coverage (>95% of genes) of induced mutations distinct from natural variants. Several independent lines displayed a novel developmental phenotype during field observation: full fertility and grain filling of all floral spikelets as opposed to only 1/3 of spikelet fertility that normally occurs in wild-type BTx623 and elite hybrid backgrounds. This phenotype, termed *multiseeded* (*msd*), greatly increases the grain number per panicle (GNP) and was manifested by several loci as determined by complementation analysis. Causative alleles were mapped by whole-genome sequencing via bulked segregant analysis. Two separate genes, *MSD1* and *MSD2*, encode a TCP (*Teosinte branched/Cycloidea/PCF*) transcription factor and Lipoxygenase enzyme, respectively, and have multiple independent nonsense or missense EMS-induced SNPs within functional domains. Transcriptomic analysis of developing panicles shows differential gene expression within a narrow timeframe; gene ontology analysis and hierarchical clustering of differentially expressed transcripts revealed an enrichment of genes involved in the jasmonic acid (JA) biosynthetic and signaling pathways. Further investigation demonstrated that the *MSD1* transcription factor could directly regulate *MSD2* and that *msd1* and *msd2* had altered bioactive JA levels in developing floral meristems, resulting in complete gynoecium and androecium formation observed in *msd* mutants. Exogenous application of JA rescued the *msd* phenotype, confirming the role of *MSDs* and the hormone in sex organ development. Our results demonstrate the power and speed of curating agriculturally significant sorghum lines such as increased GNP cultivars through genomic, molecular, and genetic avenues.

Gene / Gene Models described: *MSD1*, *MSD2*; SORBI\_3007G135700

Funding acknowledgement: United States Department of Agriculture (USDA)

P203

## *grassy tillers1* (*gt1*) and *ramosa3* (*ra3*) act together to suppress carpels in the tassel and ear

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Growth suppression is a fundamental process in the development of plant form. Early in maize floral development, carpels are suppressed by programmed cell death in the tassel and in lower ear florets. Several hormones and transcription factors regulate maize carpel suppression, but the explicit molecular mechanism is still unknown. One regulator of carpel suppression is *grassy tillers1* (*gt1*), a class 1 HD-ZIP transcription factor that partially suppresses carpels in the tassel. To identify regulators acting with *gt1* to suppress carpels, we conducted an EMS mutagenesis screen on *gt1* mutant individuals. In this screen, we identified 5 families that segregated an enhanced *gt1* mutant phenotype that we call *rapunzel* (*rzl*). *rzl3* and *rzl4* mutants are allelic and grow silks in all tassel florets and all lower florets in the ear. Using bulk segregant analysis coupled to whole genome sequencing, we found that *rzl4* encodes *ramosa3* (*ra3*). *ra3* encodes a trehalose-6-phosphate phosphatase (TPP) with known roles in suppressing tassel and ear branching. Curiously, *ra3* mutants do not have derepressed carpels in the tassel or ear. Current research in the lab is focused on understanding the molecular mechanisms underlying this unexpected synergistic phenotype in *gt1 ra3* double mutants.

Gene / Gene Models described: *gt1*, *ra3*; Zm00001d028129, Zm00001d022193

Funding acknowledgement: United States Department of Agriculture (USDA)

P204

## Growth and development of *Setaria viridis* (Poaceae) under normal and shaded light regimes

(submitted by Qing Li <[qing.li@okstate.edu](mailto:qing.li@okstate.edu)>)

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Plants rely on photosynthesis to gain energy for growth, yet most plants are found with other plants, and therefore need to deal with varying degrees of shading. Shading by plants affects both the spectrum (especially red (R) to far-red (FR) ratio) and the amount of photosynthetically active radiation (PAR, 400-700 nm). The shade avoidance syndrome has been well studied in the dicot species *Arabidopsis thaliana*, but relatively little in grasses. In addition, few studies have investigated the development trajectory of plants under different shading treatments. The goal of this study is to understand the effect of light quality and quantity on shade responses in the C4 grass, *Setaria viridis*. To achieve this, plants were grown under combinations of high or low light intensity, paired with high or low R: FR (mimicking sunlight or shade respectively) in continuous light. Both top view and side view images were taken at 15 min intervals during the growth of the plants, with a custom-designed imaging system that used individual Raspberry Pi NOIR cameras dedicated to each plant. Custom OpenCV scripts were written to extract relevant plant trait information from the images, including blade vertex coordinates, total leaf area involved in photosynthesis, and height. Our novel approach allows us to capture the behavior of individual organs, rather than more commonly used measures that focus on overall plant shape. By flowering time, plant shading (both simulated by the reduction of R: FR and by reduced light intensity) resulted in a significant increase of height, number of tillers, and biomass of plants. Developmental analysis also revealed changes in leaf orientation and increased variation in leaf position and leaf movement in plants grown under low R: FR.

Funding acknowledgement: National Science Foundation (NSF)

P205 

## High jasmonic acid levels characterize the juvenile phase of maize shoots

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During the juvenile phase of maize, the first 4-5 leaves differentiate with a suite of characteristics that distinguish them from leaves that differentiate later, when the plant is an adult. To learn how these differences arise we previously compared gene expression patterns in plastochron 6-staged primordia of leaves 1-12. Primordia of leaves destined to have juvenile traits upregulated genes involved in the light reactions of photosynthesis, in abiotic stress responses, as well as miR156, the master regulator of juvenility in angiosperms. Expression patterns of genes responsible for the production of jasmonic acid prompted us to hypothesize that high JA is required for the duration of the juvenile phase, and indeed, treatment with JA prolonged the juvenile phase in a dose-dependent manner, including delaying the decline in levels of miR156. We have used LC-MS to quantify JA and methyl JA, as well as GA, known for its role in promoting adult differentiation, in selected leaves at various plastochrons. While higher in all juvenile samples, JA was highest in leaf 1, peaking at plastochron 8. GA showed a reciprocal pattern, higher in adult samples than in juvenile leaf primordia. Levels of JA, meJA, and GA in phase change mutants provide insight into how these hormones influence vegetative phase change. Patterns of gene expression were consistent with a model in which the stresses of germination result in increasing JA levels, followed by reduction in miR156-targeted transcripts to establish the juvenile phase in maize.

Funding acknowledgement: National Science Foundation (NSF)



**P206**

## **How leaves grow wide: NARROWSHEATH controls mediolateral outgrowth of lateral organs**

(submitted by Brianne Conlon <[brc82@cornell.edu](mailto:brc82@cornell.edu)>)

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The mechanisms whereby lateral organ initial cells are organized from the peripheral zone of the shoot apical meristem (SAM) are poorly understood. The maize gene NARROWSHEATH1 (NS1)/WOX3 is expressed at the marginal boundary of leaf founder cells in the SAM and in young leaf primordia, where it mediates mediolateral outgrowth. To investigate the mechanisms of NS1 function, we used ChIP-seq of NS1 followed by laser-microdissection RNAseq of ns mutant and wild type primordial margins to identify gene targets that are bound and modulated by NS1. In a comparative approach, ChIP-seq was also performed on the Arabidopsis WOX3 paralog PRESSED FLOWER1 (PRS1), to identify conserved mechanisms of founder cell recruitment and primordial outgrowth in maize and Arabidopsis. These data, combined with microscopic analyses of cell division dynamics, reverse genetic analyses of homologous NS1/WOX3 target genes in Arabidopsis, and NS1 overexpressing plants in maize, suggest that NS1/WOX3 controls mediolateral outgrowth by direct repression of growth inhibitory genes and indirect promotion of cell division in primordial leaf margins. Intriguingly, the homologous WOX genes WUS1 and WOX5 are expressed in the organizing centers of the Arabidopsis SAM and root meristem respectively, whereupon these protein products traffic to adjoining cells to activate stem cell identity non-autonomously. In contrast, our previous data revealed that PRS1/WOX3 does not traffic suggesting that, in combination with these latest data, there is domain specific function where NS1/WOX3 stimulates primordial cell division in the same margin initial cells where it is transcribed.

Gene / Gene Models described: *ns1*; GRMZM2G069028

Funding acknowledgement: National Science Foundation (NSF)

**P207**

## **Improved vectors and genetic switches for gene editing technologies**

(submitted by David Wright <[wrightd@iastate.edu](mailto:wrightd@iastate.edu)>)

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The Iowa State University Plant Transformation Facility (PTF) provides crop plant transformation services for maize (Hi II and B104), Soybean (Williams 82, Thorne and Jack) and rice (Nipponbare and Kitaake). Services include generation of transgenic callus, plantlets and seed for maize, plantlets and seed for soybean and plantlets for rice. Services are for research or teaching purposes and include gene editing, and classic transformation projects. The facility has a new website and on-line ordering system to better serve our clients (<https://www.biotech.iastate.edu/biotechnology-service-facilities/plant-transformation-facility/>). The PTF focuses on improving transformation protocols, advanced vector designs and enhanced small molecule activated genetic switches based on published work from the laboratories of David Liu and David Wood. The switches are intended to improve the safety and accuracy of gene editing technologies and the work is funded by the Iowa State University Crop Bioengineering Center.

Funding acknowledgement: Iowa State University Crop Bioengineering Center

P208 

## **INDETERMINATE DOMAIN (IDD) family genes - the candidate regulators of early leaf development in C4 grasses perturb root development when mutated in *Setaria viridis***

(submitted by Dhineshkumar Thiruppathi <[dthiruppathi@danforthcenter.org](mailto:dthiruppathi@danforthcenter.org)>)

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C4 grasses are major contributors to the world's food supply. Their highly efficient method of carbon fixation is a unique adaptation that combines close vein spacing and distinct photosynthetic cell types. Despite its importance, the molecular genetic basis of C4 leaf development is still poorly understood. The INDETERMINATE DOMAIN (IDD) family of transcription factors is a candidate regulator of C4 leaf anatomy, based on a comparative transcriptomic study of C4 and C3 grass species. We tested whether IDD2 and its paralog IDD11 affected leaf anatomy in a C4 model grass *Setaria viridis*. Knockout mutants were generated using CRISPR-Cas9 technology, which altered expression of SvIDD2 and SvIDD11. None of the genes were individually or together sufficient to alter C4 vein patterning or cell-type differentiation in *Setaria*, eliminating them from a list of potential C4 leaf patterning regulators. Intriguingly, we found that mutations of both SvIDD2 and SvIDD11 resulted in plants with substantially shorter roots than wildtype, a phenotype similar in plants with altered expression of IDDs of *Arabidopsis* and rice. Expression patterns of SvIDD2 and SvIDD11 were largely root-specific based on qRT-PCR and promoter-reporter (GUS and GFP fusions) studies. Both transient and stably expressed GFP-tagged SvIDD2 or SvIDD11 confirmed their localization to the nucleus. A yeast-2-hybrid assay showed that, like its sequence orthologs in *Arabidopsis*, SvIDD2 interacts with *Setaria* ortholog of *Arabidopsis* SHORTROOT, a master regulator of root development. Thus, SvIDD2 and SvIDD11 function in root development. Future root transcriptome analysis will test whether known and/or novel root developmental genes are affected in mutant and overexpressing lines. In addition, 3D root imaging and physiological experiments will shed more light on the roles of SvIDD2 and SvIDD11 in regulating root system architectural traits in *Setaria*. Such detailed functional information will open possibilities for translational studies that manipulate equivalent root architectural traits in crops controlled by orthologous IDD genes.

Funding acknowledgement: National Science Foundation (NSF)

P209 

## **Inflorescence induction causing physiological variation in *diploperennis***

(submitted by Lillian Hislop <[lmhislop@wisc.edu](mailto:lmhislop@wisc.edu)>)

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*Diploperennis* is a short-day perennial variety of teosinte, the evolutionary ancestor of maize. In recent crossing attempts at the University of Wisconsin-Madison, inflorescence was induced in *diploperennis* by artificial shortening day length. The induced individuals exhibited unique physiological variations compared to their uninduced counterparts, more resembling the taller, less dense structure of maize. This poster will propose follow up research to determine the cause of these physiological changes, whether due to shortened day length, the constrictive environment, or the induction itself. The outcome of this research will reveal information about teosinte, how researchers might more efficiently induce flowering, and possibly new information about maize's evolution.

P210

## Interactions of boron, phytohormones, and meristem maintenance pathways during vegetative and reproductive development in maize

(submitted by Michaela Matthes <[matthesm@missouri.edu](mailto:matthesm@missouri.edu)>)

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The essential micronutrient boron plays a well described stabilizing role in the plant cell wall. However, there is increasing evidence suggesting additional functions of boron beyond the cell wall. Plants have to take up boron from the soil with the help of transport proteins like TASSEL-LESS1 (TLS1) in *Zea mays* (maize). Our lab previously cloned and characterized *tls1* and showed that one of the first defects in the boron deficient *tls1* mutant is a reduction in meristem size, indicating defects in maintaining meristematic cells. To assess a functional role of boron in meristem development, we analyzed double mutants of *tls1* with different mutants of the CLAVATA-WUSCHEL (CLV-WUS) pathway that regulates meristem size. Specifically, we analyzed vegetative, tassel (male inflorescence), and ear (female inflorescence) phenotypes. We observed contrasting effects on the *tls1* reproductive defects when crossed with different mutants of the CLV-WUS pathway. Due to the importance of hormonal pathways during meristem development, we also analyzed double mutants between *tls1* and mutants in auxin biosynthesis, as well as auxin and cytokinin signaling. Our data shows that boron deficiency symptoms in *tls1* are enhanced with reduced auxin levels and that altered cytokinin signaling partially suppresses the reproductive defects seen in *tls1*. These results are further supported by confocal microscopy of auxin and cytokinin marker lines in *tls1* meristems. The results from our study are particularly exciting because they indicate new roles of boron in meristem maintenance and hormonal signaling pathways that can be linked to each other. Specifically, the observed suppression of the *tls1* reproductive defects by *thick tassel dwarf1* (*td1*), the CLV1 ortholog, implicate additional roles of boron beyond the cell wall and resolve a long-standing controversy in the field.

Funding acknowledgement: United States Department of Agriculture (USDA)

P211

## Investigating the role of the adult maize leaf cuticle in providing pathogen resistance

(submitted by Albert Nguyen <[amn026@ucsd.edu](mailto:amn026@ucsd.edu)>)

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The plant cuticle is a waxy, hydrophobic layer found on all aerial non-woody plant tissue, which serves as a physical barrier protecting the plant from environmental stresses including pathogen infection, dehydration and UV radiation. Studies on the plant cuticle in various plant model systems show differences in ultrastructure and chemical composition across different plant species, and even within a species, depending on many factors: organ identity, developmental stage and growth conditions. Little functional analysis has been performed to characterize the cuticle's relationship to pathogen resistance in adult *Zea mays*, the highest grossing industrial and agricultural crop in the United States, thus leaving the agronomic impact of the cuticle on the adult-stage plant health largely unknown. Maize glossy mutants have been identified to show defects in cuticle production—examples include lines with impaired levels of lipid biosynthesis or wax transport proteins, which make them effective tools to study the cuticle's impact on pathogen resistance.

In this study, we take glossy mutants and observe their differences in the early stages of pathogen resistance to *Cochliobolus heterostrophus*, the causal necrotrophic agent in Southern Leaf Blight. After establishing methods to observe and quantify GFP-labelled *C. heterostrophus* infection stages on adult plants, which include fungal adhesion, and long-term visual lesion formation, we detected differences in *C. heterostrophus* performance among a few members of a panel of tested glossy mutants by utilizing epifluorescence microscopy and GFP quantification. Biochemical studies to attribute these changes to cuticle composition were performed by Dr. Isabel Molina's research group using gas chromatography/mass spectrophotometry. Further steps to analyze the cuticle-fungal penetration relationship and cuticle ultrastructure are ongoing.

Funding acknowledgement: National Science Foundation (NSF)

P212

### Lab to field comparison of maize growth

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To identify the basis for the high attrition rate in the translation of molecular knowledge generated in controlled lab conditions to applications in the field, we performed a comparative study in maize between the growth chamber, greenhouse and field conditions at the phenotypic and molecular level. Although the absolute values vary tremendously, identical traits significantly correlate across conditions. The growth of juvenile leaves measured in growth chamber and greenhouse was related to early plant growth rate in the field while the mature leaf lengths and biomass determined in the greenhouse highly correlated with plant biomass of field grown plants. Comparison of the transcriptome revealed a set of genes that were robustly differentially expressed between the lab and the field, in which shade avoidance and senescence were major responses. In addition, many genes known to be involved in abiotic stress and development were extremely differentially expressed, which may explain the troublesome nature of translating yield stability traits. Our data show that a more comprehensive understanding of the difference between the lab and the field is imperative to ensure a higher success rate in translating findings in the lab into applications.

P213 

### Maize and human RNA binding motif protein 48 have an evolutionarily conserved essential role in U12-dependent intron splicing

(submitted by Shailesh Lal <[lal@oakland.edu](mailto:lal@oakland.edu)>)

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U12-type or minor introns are found in the vast majority of multicellular eukaryotes, including both plants and animals, and are spliced by a distinct minor spliceosome. Although U12 introns constitute less than 0.5% of all introns, minor intron containing genes (MIGs) have been shown to have roles in growth and development. Aberrant splicing of U12-type introns in human hematopoietic stem cells disrupts differentiation of the myeloid lineage. Recently, we reported that maize RNA Binding Motif Protein 48 (RBM48) is required for U12-type intron splicing. Mutants of maize *rbm48* display abnormal endosperm cell differentiation and proliferation, and show genome-wide aberration of primarily U12-type intron splicing (Bai et al., 2019). An ortholog of maize RBM48 exists in humans. To investigate whether the role of RBM48 in U12 splicing is conserved between maize and humans, we generated a CRISPR/Cas9-mediated RBM48 functional knockout (RBM48 FunKO) in human K-562 cells. Using comprehensive transcriptome profiling, we demonstrate the role of RBM48 in U12 splicing is conserved between maize and humans. By comparative RNA-seq analysis, we have identified candidate MIGs that display aberrant splicing of U12-type introns and are reciprocal best-match homologs in maize and humans. The conservation of impacted MIGs between maize *rbm48* mutants and human RBM48 FunKO cells points to potentially similar roles in normal growth and development. Mutations in several of these MIGs cause developmental defects in both plants and animals. For example, disruption of the human Stable Maintenance of Chromosome 3 (SMC3) gene and the orthologous Arabidopsis TTN7 gene have been reported to cause severe developmental defects in both species. Our data indicates that RBM48 is required for efficient U12 splicing and that affected MIGs are predicted to disrupt normal growth and development in diverse eukaryotes.

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

P214

## **Maize *Dek33* encodes a pyrimidine reductase in riboflavin biosynthesis essential for oil body formation and ABA biosynthesis during seed development**

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The maize (*Zea mays*) classic mutant *defective kernel 33* (*dek33*) produced defective and occasionally viviparous kernel phenotype. In this study, we cloned *Dek33* by positional cloning and found that it encodes the pyrimidine reductase in riboflavin biosynthesis. In *dek33*, a single base mutation (G/A) in the C-terminal COG3236 domain caused a premature stop codon (TGA), producing a weak mutant allele with significant reduction of truncated DEK33 protein and riboflavin content. The *dek33* mutation significantly affected oil body formation and suppressed cell proliferation. The *dek33* mutation also disrupted ABA biosynthesis, resulting in less carotenoids and ABA content, which is responsible for the viviparous embryo. In addition, our results indicated that the COG3236 domain is important for the protein stability of DEK33. The yeast two-hybrid experiments identified several proteins that interact with DEK33, including RGLG2 and SnRK1, suggesting possible post-translational regulation to DEK33 stability. This study provided comprehensive insights into cellular responses due to impaired riboflavin biosynthesis during maize seed development.

Gene / Gene Models described: *dek33*; GRMZM2G090068

Funding acknowledgement: National Key Research and Development Program of China, Ministry of Science and Technology of China, National Natural Sciences Foundation of China

P215

## **Maize *ethylene insensitive3-like* genes regulate plant architecture**

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The phytohormone ethylene is a key regulator of plant growth and development, and is a pivotal stress response signal. The genetic basis of ethylene biosynthesis, perception and signaling is largely informed by studies in *Arabidopsis thaliana*; however, natural variation of ethylene response across accessions of *Arabidopsis* has not been reported. Furthermore, it is unclear how broadly conserved ethylene-regulated developmental and physiological pathways are between *Arabidopsis* and monocot species, such as maize. We set out to understand the functional conservation of ethylene signaling genes between *Arabidopsis* and maize, and to explore natural variation in ethylene response. We characterized mutations in *ZmETHYLENE INSENSITIVE3-LIKE* (*ZmEIL*) genes that are homologs of *AtEIN3*. Higher-order *Zmeil* mutants display developmental and stress-related phenotypes that are unlike phenotypes reported for *Atein3* mutants. These observations suggest that ethylene signaling pathways of *Arabidopsis* and maize have diverged and/or the downstream networks that respond to ethylene are markedly different. Current experiments are testing these hypotheses. We developed an etiolated germination assay to evaluate growth response to the ethylene precursor ACC and used it to screen the maize Nested Association Mapping founder lines for response in coleoptile, mesocotyl and root. In the presence of ACC, some germinant seedlings displayed overall reduced growth compared to control siblings, some showed no change in growth, and other inbred lines exhibited more growth relative to control. Additionally, this work uncovered tissue-specific variation in response to ACC. These observations indicate diverse maize inbred lines exhibit significant tissue-specific hyper-, hypo- and insensitive responses to ethylene. Work aimed at understanding the genetic architecture that underlies natural variation to ethylene in maize is ongoing. Taken together, our findings indicate *ZmEIL* genes are major regulators of maize development and growth, and maize displays significant natural variation in ethylene sensitivity.

Funding acknowledgement: National Science Foundation (NSF)

P216 

## Maize genes at the intersection of development and immunity

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Maize development is controlled by a number of crucial signalling networks and can be heavily influenced by external stimuli including ambient temperature and the presence of pathogens. The dominant developmental mutant, *Liguleless narrow* (*Lgn-R*), is caused by a point mutation in a receptor like kinase. Heterozygous mutants in a B73 background are short with narrow leaves and struggle to develop inflorescences. However, the same mutation in a Mo17 background leads to a phenotype that is difficult to distinguish from non-mutant siblings. Furthermore, the penetrance of the phenotype is temperature sensitive. When *Lgn-R* B73 plants are grown in hotter temperatures they die and *Lgn-R* Mo17 plants develop a stunted phenotype. Recombinant mapping combined with association analysis allowed us to map a Mo17 modifier of *Lgn-R* named *Sympathy for the ligule* (*Sol*). *Sol* is a maize homolog of the Arabidopsis gene ENHANCED DISEASE RESISTANCE4 (EDR4), and, like EDR4, it increases in expression in response to exogenous treatment by PAMPs, including flg22 and chitin. *Sol* is also increased in expression in the *Lgn-R* B73 background but its expression is comparable to non-mutant levels in a near isogenic line that includes a copy of *Sol* from Mo17. RNAseq and phosphoproteomic analyses indicate activation of an immune response in our severe mutants. Based on the pleiotropy, background dependence, and temperature sensitivity of the *Lgn-R* phenotype as well as the identity and behavior of *Sol* we are putatively claiming that the mutation in *Lgn* triggers an autoimmune response in B73, leading to many of its downstream developmental defects. In the Mo17 background *Sol* is able to mitigate this downstream signalling in an unknown manner and the autoimmune response is suppressed.

Gene / Gene Models described: *lgn*, *sol*; GRMZM2G134382, GRMZM2G0725262

Funding acknowledgement: University of California, Berkeley



P217

## Maize unstable factor for orange1 plays a role in carbohydrate accumulation and kernel development

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The maize *Unstable factor for orange1-1* (*Ufo1-1*) is a spontaneous dominant allele that modifies the expression of *pericarp color1* (*p1*) resulting in over-accumulation of reddish-orange flavonoid pigments in kernel pericarp, cob glumes, and other vegetative tissues. We have recently identified the lesion responsible for *Ufo1-1* phenotypes and cloned the *ufo1* gene. It encodes a novel protein with orthologues in sorghum, setaria and rice but its function is not known. It is highly expressed in developing maize kernel's basal endosperm transfer layer (BETL) and conducting zone (CZ). The BETL and CZ are specialized tissues that allow transport of photosynthates. The *Ufo1-1* kernels have reduced total starch and increased soluble sugars and reduced seed weight. Intestinally, the *Ufo1-1* transcriptome shows abnormal expression of essential genes involved in sugar and hormone homeostasis during endosperm development. In addition to the *Ufo1-1*, a transposon insertion (*ufo1-Ds*) loss of function allele is being characterized. The *ufo1-Ds* plants also show abnormalities in sugars and hormones accumulation. We are thus investigating the interaction of *Ufo1* with maize genes essential for kernel development: *basal endosperm transfer layer 1* (*bet1*), *viviparous1* (*vp1*), *floury2* (*fl2*), *globulin3* (*glb3*) and *responsive to aba17* (*rab17*). Transient expression of UFO1-GFP fusion protein in *Nicotiana benthamiana* shows nuclear and nucleolar localization indicating its importance in regulating major biological processes. Further, the up regulation of GO term for 'response to stress' and down regulation of GO terms for 'ribosome biogenesis' provide hints of its possible function. Our ongoing research on understanding the role of *ufo1* during seed development will be presented.

Gene / Gene Models described: *bet1*, *vp1*, *fl2*, *glb3*, *rab17*; GRMZM2G053177

Funding acknowledgement: National Science Foundation (NSF), Indian Council of Agricultural Research (ICAR), AES project PEN04613

P218

## Maize VKS1 is essential for early endosperm development by regulating mitosis and cytokinesis

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Cell number is a critical factor that determines maize kernel size. Rapid mitotic divisions in early endosperm development produce most cells comprising the starchy endosperm; however, the mechanisms underlying early endosperm development remain largely unknown. We isolated a previously undescribed maize mutant that shows a varied-kernel-size phenotype (*vks1*). *Vks1* encodes ZmKIN11, which belongs to the kinesin-14 subfamily and is predominantly expressed in early endosperm development. VKS1 localizes to the nucleus and microtubules and plays key roles in free nuclei migration in the syncytium as well as in mitosis and cytokinesis in early mitotic divisions. Absence of VKS1 has relatively minor effects on plants but causes deformities in spindle assembly, sister chromatid separation and phragmoplast formation in early endosperm development, thereby resulting in reduced cell proliferation. Severities of aberrant mitosis and cytokinesis within individual *vks1* endosperms differ, thereby resulting in varied kernel sizes. Our discovery highlights VKS1 as a central regulator of mitosis in early maize endosperm development and provides a potential means for future yield improvement.

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P219

### Map-Based Cloning of *leafy\** in Maize

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Shoot architecture is incredibly diverse across plant species and directly relates to the production of the fruit and grain crops we eat. Some key features of this variation are yield, biomass, harvestability and seed production. Shoot and inflorescence architecture are key determinants of these traits. Maize has two inflorescences, the tassel and the ear, both of which are important for yield. *leafy\** is a previously undescribed mutant in maize that behaves recessively and results in shortened upper internodes, a shorter overall plant and an intriguing tassel phenotype ranging from normal looking to partially feminized and with reduced branching. The *leafy\** gene's location is currently unknown as well as its molecular function. To help locate this gene we are using map-based cloning, a powerful technique that uses genetic mapping to define a progressively narrower chromosomal area containing the mutant locus until the mutation is identified. This is done by generating a mapping population, extracting DNA from wild-type and mutant individuals, genotyping with markers, identifying linked markers, genotyping to identify recombinants and then finally fine mapping to narrow the interval where the candidate genes are located. The region where *leafy\** is located has been narrowed down to 21 candidate genes, in a chromosomal interval without other, known mutants. Our long term goal is to locate the *leafy\** gene in maize, further characterize the mutant phenotype and better understand the molecular mechanisms of this gene.

Funding acknowledgement: National Science Foundation (NSF)

P220

### Mapping and characterizing *large scutellar node1* (*lsn1*) in maize

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A recessive maize mutant, *large scutellar node1* (*lsn1*), was isolated and described to have aberrant seedling development and severe vascular defects (Landoni et al., 2000). The *lsn1* seedling is characterized by a short primary root with a fasciated or flattened tip and a bulging scutellar node (the stem node on the embryo axis that connects the scutellum to the radicle). The present study aims to further characterize the *lsn1* mutant using genetic approaches and identify the causative gene using genomic and bioinformatic approaches. The segregation of the *lsn1* mutant was analyzed in five different maize genetic backgrounds: B73, A632, W22, Oh43, and Mo17. The segregation ratio of *lsn1* in the B73 background is consistent with a recessive phenotypic ratio of 3:1, but less consistent in other backgrounds, which could indicate the presence of genetic modifiers in these backgrounds. The root and vascular defects of *lsn1* point to a possible connection with the plant growth hormone, auxin. In order to investigate this connection, *lsn1* was crossed with an auxin biosynthesis mutant, *vanishing tassel2* (*vt2*) and with an auxin transport mutant, *barren inflorescence2* (*bif2*). Preliminary observations of the progenies reveal changes in the severity of the *lsn1* phenotype in combination with *vt2* and *bif2* which may indicate that the *lsn1* gene and auxin have overlapping functions. In order to identify the causative gene, the *lsn1* locus was mapped using bulked segregant analysis and next generation sequencing. Identification and characterization of the causative mutation of *lsn1* will lead to a better understanding of the role of *lsn1* in mechanisms that regulate organ development in maize.

Funding acknowledgement: National Science Foundation (NSF)

P221

## Meiosis-Associated Argonautes (MAGO)s are required for germline development of maize under heat stress

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Maize has a discrete group of 21- and 24-nt phased small-interfering RNAs (phasiRNAs), which accumulate in pre-meiotic and meiotic anthers respectively. However, the precise function of these RNAs is currently unknown. We have identified two Meiosis-Associated Argonaute (MAGO) proteins, which specifically accumulate in the epidermis of pre-meiotic anthers and in developing meiocytes, and are necessary for meiotic progression. MAGO proteins are associated with a large amount of phasiRNAs and that the mobility of these non-coding RNAs is required for male fertility. In addition, fertility defects in MAGO mutants are enhanced by temperature stress, suggesting that MAGO proteins and phasiRNAs may be involved in protecting the male germline from damage directed by environmental stress.

Funding acknowledgement: BBSRC

P222

## Microautophagy of storage proteins in maize aleurone cells

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In the cereal endosperm, starchy endosperm cells accumulate storage proteins (mostly prolamins) and starch whereas the peripheral aleurone cells store oils, storage proteins, and specialized metabolites. Although both aleurone and starchy endosperm cells synthesize prolamins, they employ very different pathways for their subcellular trafficking. Starchy endosperm cells accumulate prolamins in protein bodies within the endoplasmic reticulum (ER), whereas aleurone cells deliver prolamins to vacuoles *via* an autophagic mechanism that does not depend on the canonical ATG8 (AUTOPHAGY RELATED 8)-conjugation pathway. We found that the prolamins accretions in the ER of aleurone cells come in close contact with the vacuolar membrane and then are engulfed directly into vacuoles *via* microautophagy. Microautophagy is the least characterized form of autophagy at both cellular and molecular levels. In plants, the molecular machinery orchestrating microautophagy of storage proteins is completely unknown but does not appear to depend on the core ATG components. We conducted RNA-sequencing studies of aleurone and starchy endosperm tissues at 18 and 22 days-after-pollination and performed mass spectrometric analyses on vacuolar membrane-enriched fractions of aleurone cells in attempts to identify protein factors mediating microautophagy of storage proteins. We are currently examining the subcellular localization and function(s) of these candidates as possible microautophagy regulators in maize.

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

P223

***necrotic upper tips1* is a floral specific NAC transcription factor that ensures sufficient water movement and xylem cell wall integrity**

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Maintaining sufficient water transport during flowering is essential for proper organ growth, fertilization and yield. When water deficits coincide with flowering they result in severe developmental consequence such as leaf wilting, tassel browning and sterility, a condition known as “tassel blasting.” Thus, during the floral transition special mechanisms must exist to promote sufficient water transport to the growing floral apex. In order to understand the genetic mechanisms underlying this process, we have identified a mutant from an *Activator (Ac)* transposon screen, *necrotic upper tips1 (nut1)*, that mimics tassel blasting. The *nut1* mutant phenotype is evident only after the floral transition, and early vegetative development is normal. The floral nodes of *nut1* mutants have difficulty moving water as shown by dye uptake and movement assays. Plastic sections, cryofracture SEM and TEM of *nut1* vasculature show defects in protoxylem vessel thickness and integrity, providing the basis for the mutant phenotype. The *nut1* mutant is caused by an *Ac* insertion into the coding region of a *NAC* transcription factor gene. Wildtype revertants and stable deletion derivatives were isolated, confirming the identity of the gene. *nut1* is expressed only after the floral transition in the root, stem and leaf sheath, but not in meristematic tissue and leaf blade. Immunolocalization experiments using a NUT1 specific antibody showed that the protein is transiently expressed in initiating protoxylem cells and disappears after xylem maturation. Using DAP-seq and ChIP-qPCR coupled with RNA-seq, NUT1 downstream targets were identified and found to function in cell wall biosynthesis, apoptosis, and maintenance of xylem cell wall thickness and strength. These results show that unique transcription factors function within specific vascular compartments to maintain xylem vessel integrity during periods of high water movement.

Funding acknowledgement: National Science Foundation (NSF)

P224

## New insights into maize ear development using single cell (sc)RNA-Seq

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Maize productivity depends on inflorescences, whose development requires a programmed series of meristem fate decisions involving communication between different cell populations. An understanding of ear development requires insight into the full diversity of cell types and developmental domains. However, these are classified mainly by morphology, as well as by insights from classical genetics, but this is limited by genetic redundancy and pleiotropy. Single cell transcriptome profiling of the maize ear can provide high-resolution genome wide transcriptional signatures of specific cell types, and identify new developmental domains.

We isolated single cells from ear primordia by protoplasting, and used the high-throughput 10X Genomics Chromium platform to profile >10,000 individual cells. We detected expression from 28,254 genes in total, with an average of 2,938 transcripts detected per cell. Graph-based clustering partitioned cells based on their transcriptomes into 18 groups. Many groups were defined by known markers, such as an L1/epidermal group, marked by *OUTER CELL LAYER* genes, an L2 meristem group, marked by *KNOTTED1*, a primordium group, marked by *YABBY* genes, and a vasculature group, marked by *RAN BINDING PROTEIN2*. Each group contained an additional ~20-500 new candidate cell type or domain specific markers. We validated our findings using Fluorescence Activated Cell Sorting (FACS) of reporter lines, such as *pYABBY14-TagRFPt*, and the majority of enriched transcripts showed concordant differential gene expression patterns in our scRNA-seq analysis. We also localized each cell population using *in situ* hybridization for selected marker genes, and found specific, spatially restricted markers in each group. Strikingly, we also identified novel markers for specific developmental domains, such as meristem branching sites.

Collectively, we demonstrate that scRNA-seq is a powerful tool to predict genome wide gene expression domains for tens of thousands of maize ear inflorescence cells, and identified hundreds of new candidate regulators of maize inflorescence development.

Gene / Gene Models described: *ZmHDZIV4\_OCL4*, *ZmHDZIV3\_OCL3*, *ZmHDZIV5\_OCL5*, *ZmHDZIV1\_OCL1*, *kn1*, *Zmyabby15*, *Zmyabby14*, *Zmyabby9*, *Zmyabby10*, *RANBP2*; GRMZM2G123140, GRMZM2G116658, GRMZM2G130442, GRMZM2G026643, GRMZM2G017087, GRMZM2G529859, GRMZM2G005353, GRMZM2G074543, GRMZM2G167824, GRMZM2G094353

Funding acknowledgement: National Science Foundation (NSF)

P225

## **Opaque-2 regulates a complex gene network associated with cell differentiation and storage functions of maize endosperm**

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Endosperm is a filial seed structure that provides nutrients and signals essential for embryogenesis and seedling germination. Development of the cereal endosperm involves biological processes of cell differentiation that enable nutrient uptake from maternal tissues, accumulation of storage products, and their utilization during germination. However, little is known about the mechanisms that link cell differentiation processes with genes controlling storage product synthesis and deposition. Here, we used a coupled RNA sequencing (RNA-Seq) and chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) approach to identify genes directly or indirectly regulated by Opaque-2 (O2) in the maize endosperm. We identified 1,863 genes differentially expressed between wild-type B73 inbred line and an O2 mutant in B73 background (B73o2) at 15 days after pollination (DAP). By comparing the binding results obtained through ChIP-Seq with differentially expressed genes in B73 and B73o2, we identified 186 putative direct O2 targets and 1,677 indirect targets that encode a broad set of gene functionalities. Analysis of the temporal expression patterns of a subset of O2 targets revealed at least two distinct modes of O2-mediated gene activation. We also show that two O2-activated transcription factor genes, bZIP17 and NAKED ENDOSPERM2 (NKD2), which can in turn co-activate other O2 network genes with O2. Collectively, our results provide insights into the complexity of the O2-regulated network and its role in regulation of cell differentiation and function in maize endosperm.

Gene / Gene Models described: *o2*, *bzip17*, *nkd2*; GRMZM2G015534, GRMZM2G103647, GRMZM5G884137

Funding acknowledgement: National Science Foundation (NSF)

P226

## **Ovary abortion is prevalent in diverse maize inbred lines and is under genetic control.**

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Grain yield in maize is a product of individual kernel weight and kernel number; hence, factors affecting these components have received substantial attention. The potential number of kernels is determined by the number of mature florets on the ear inflorescence. Each floret contains an ovary with a single megametophyte that can develop into a kernel. Failure of kernels to develop from ovaries reduces grain number on the ear, and kernel number, rather than kernel weight, has the larger impact on yield. Therefore, strategies that maximize kernel number could translate into increased yield. Here, we describe an important characteristic for maize productivity. Despite the fact mature maize ears are typically covered with kernels, we find that 35% to 40% of ovaries fail to give rise to mature kernels. Non-developed ovaries degenerate while neighboring fertilized ovaries produce kernels that fill the ear. The loss of kernels, which we term kernel abortion, occurs throughout the ear, not just at the tip. We show that the fraction of aborted ovaries/kernels is genetically controlled and varies widely among maize lines. Low abortion genotypes are rare. Reducing or eliminating ovary abortion could substantially increase yield, making this characteristic a new target for selection in maize improvement programs.

Funding acknowledgement: National Science Foundation (NSF)



P227

## Phenotypic comparison of suppressor of sessile spikelet (*Sos*) mutants in maize

(submitted by Connor Nordwald <[clnqrc@mail.missouri.edu](mailto:clnqrc@mail.missouri.edu)>)

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Maize and other grasses provide a major source of food across the globe. Maize has evolved to produce paired spikelets as opposed to the single spikelet structure that is seen in other grasses such as rice and wheat. This is due to differences in the development of the maize inflorescence. Maize spikelets form in pairs from the inflorescence meristem (IM) which produces spikelet pair meristems (SPM). The SPM then split into two spikelet meristems (SM). In order to understand how this happens, the semi-dominant Suppressor of Sessile Spikelets mutations (*Sos2* and *Sos3*) are being studied and compared. Heterozygous *Sos2* and *Sos3* mutants produce single spikelets in the tassel and the ear indicating a defect in SPM development. Homozygous *Sos2* plants produce an ear and tassel with only a few spikelets present, and *Sos3* plants have ears and tassels with barren patches indicating additional defects in SPM development. While studying these mutations new data has been collected that suggests *Sos2* and *Sos3* may be related genes. To test this hypothesis, a double mutant analysis was conducted by backcrossing both mutants with various mutants in the maize CLAVATA pathway to see if the *Sos* mutants exhibit similar genetic interactions in the meristem. The genetic responses were quantified using phenotypic data and Scanning Electron Microscopy (SEM) on immature ears. Through comparing the results of these experiments, a better understanding of how *Sos2* and *Sos3* function together or separately in meristem development will be obtained.

Funding acknowledgement: National Science Foundation (NSF)

P228 

## Priming modulation of early maize seedling performance under cold stress using vigor: A machine vision HT seedling emergence assay

(submitted by Gokhan Hacisalihoglu <[gokhan.h@famu.edu](mailto:gokhan.h@famu.edu)>)

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Emergence under cold temperatures is an important trait and therefore developing new, reliable, high throughput phenotyping tools is necessary to assist plant breeders. Seedling emergence is an important factor for yield, particularly under challenging planting conditions. In the US corn belt, maize is planted in early spring, as soon as soil temperatures are permissive to germination. At that time, temperatures often drop below normal, which can delay or even kill the seedling. Seed pre-treatments have been shown to improve germination in cold conditions in crops such as rice and cabbage, but are largely unpublished in maize. To assess the effects of pre-treatments on early maize cold tolerance, twenty-seven inbred parents of maize Nested Association Mapping (NAM) population were primed using a synthetic solid matrix and then tested for cold tolerance by incubation at 10°C for 5 days. Following the cold treatment, kernels were transferred to control conditions (24°C) and emergence was monitored using Vigor: a soil-based machine vision assay for seedling emergence. Vigor uses DSLR cameras to capture images every 30 min. Time lapsed images are streamed to CyVerse cyber infrastructure where the image stacks are analyzed by the Vigor application to determine emergence frame. Population metrics were calculated for each genotype including final emergence percentage, time to 50% emergence, and emergence rate. The cold treatment reduced total emergence of several genotypes. However, priming pre-treatment protected the sensitive genotypes allowing nearly full emergence. We also used single-kernel near infrared reflectance spectroscopy to determine seed density, weight, oil, protein, and starch for the kernels prior to planting. By combining kernel characteristics and emergence time, we found small, but highly significant correlations between the kernel and early seedling performance. Results show that our machine vision based HT phenotyping can be used in plant research and breeding applications.

Funding acknowledgement: National Science Foundation (NSF)

P229

## **Quantitative phosphoproteomics reveals novel regulators of maize defenses against biotic stress**

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Biotic stress consistently reduces annual crop yields and is expected to increase in severity as environmental stress conditions worsen. Better understanding of molecular processes regulating plant defenses against biotic attack will greatly assist efforts to enhance resistance through targeted gene editing or breeding. While defense-induced transcriptional and metabolic changes have been extensively studied in diverse species under different types of biotic stresses, rapidly occurring upstream signaling events remain less well studied, particularly in maize. Many proteins that coordinate initial signaling events aren't regulated at the transcriptional or translational levels, but are activated through rapid post-translational modifications such as phosphorylation. In an effort to better understand maize defense signaling and identify rapidly-responding regulatory components, we deployed highly sensitive nano liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) to screen for changes in phosphoproteomic patterns 10 minutes after treatment with an endogenous defense-eliciting signal, Plant Elicitor Peptide (Pep). Peps are a family of signals regulating plant defense conserved throughout higher plants, and in maize have been demonstrated to activate a broad array of defense responses and confer resistance to both insect pests and pathogens. We detected more than 150 proteins that were rapidly altered in phosphorylation state after Pep treatment, most of which have not been previously implicated in defense responses. To assess the efficacy of this method for identification of novel defense components, we selected candidate genes with available transposon insertional mutant lines or used a Virus-Induced Gene Silencing (VIGS) system for phenotypic screening. A rapid and simple screening method based on assessment of altered defense metabolism using gas chromatography revealed that perturbation of several candidate genes results in altered defense phenotypes. These results demonstrate the efficacy of phosphoproteomic data as a tool for identifying and characterizing regulatory proteins and provide novel candidates for manipulation of maize resistance to biotic stress.

Funding acknowledgement: UCSD Start-Up Funds, UCSD Cell and Molecular Genetics Training Program

P230 

## Reconstructing the transcriptional ontogeny of maize and sorghum supports an inverse hourglass model of inflorescence development

(submitted by Sam Leiboff <[sleiboff@berkeley.edu](mailto:sleiboff@berkeley.edu)>)

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The body plans of agricultural species, such as maize and sorghum are shaped by the evolution of developmental pathways. Surveys of plant, animal, and fungal tissues have uncovered an evolutionarily-conserved ‘developmental hourglass,’ where closely-related taxa have transcriptional similarity at developmental stages where a shared body plan is established. Evolutionarily distant taxa, however, exhibit a transcriptional ‘inverse-hourglass’ with the greatest differences in expression at stages where their unique body plans are formed. To understand the comparative development of maize tassels and sorghum panicles, we collected a series of individual RNAseq profiles encompassing inflorescence body plan specification in both species. Using smoothing-splines to summarize our samples, we reconstructed developmental gene dynamics from 40 B73 maize tassel and 48 BTx623 sorghum panicle transcriptomes, comprised by 5 maize stages and 4 sorghum stages. To discover new putative molecular markers of maize and sorghum development, we used random forest machine learning to evaluate inflorescence stage by RNAseq profile. Our entrained model correctly classifies published B73 tassel primordia, and is available as a public resource. To detect evolutionary forces that shape inflorescence architecture, we queried maize and sorghum against 216 eukaryotic and bacterial proteomes. High scoring alignments were used to calculate TAI as an measure of ancient gene activity. We additionally compared codon substitution rates against the *Setaria italica* genome to calculate TDI as an orthogonal measure of conserved gene activity. Both TAI and TDI identified hourglass-like stages in sorghum (branch meristem stage) and maize development (floral meristem stage). Despite close evolutionary ancestry, transcriptomic comparisons found that maize and sorghum inflorescences are most different during their hourglass-like stages of development, following an ‘inverse-hourglass’ model of development. We propose that agricultural selection for male sex specification in maize and increased grain production in sorghum may account for the rapid divergence of body plan pathways in these species.

Funding acknowledgement: National Science Foundation (NSF)

P231 

## Regulatory variation controlling architectural diversity in maize

(submitted by Andrea Eveland <[aeveland@danforthcenter.org](mailto:aeveland@danforthcenter.org)>)

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Pleiotropy is the effect a gene has on multiple phenotypic characters, and it represents a major force that shapes and constrains biological evolution. A well-established principle of developmental evolution is that genes are reused in different developmental processes leading to pleiotropic effects. The importance of *cis*-regulatory elements for adaptive evolution is thought to result from their reduced pleiotropy relative to protein-coding variants. Thus, the ability to manipulate *cis*-regulatory elements at functionally conserved pleiotropic loci would allow greater precision for engineering and/or breeding of optimal crop ideotypes. Several lines of evidence in maize suggest a common gene regulatory network functions at the boundaries of distinct lateral organs and contributes to pleiotropy between leaf angle and tassel branch number, two important agronomic traits. With this project, we aim to uncover genetic variation in pleiotropic loci and determine how that variation mediates phenotypic effects in these traits. First, we are integrating context-specific multi-omics datasets to define core regulatory modules that function at lateral organ boundaries, and promote development of morphologically distinct organs in maize. Second, we are using quantitative approaches that leverage maize diversity to explore allelic variation in these modules and how it translates to phenotype. Finally, hypotheses on function of *cis*-regulatory variants controlling pleiotropic loci will be tested using functional genomics approaches, including genome editing. These integrated analyses will define regulatory loci that control architectural variation across maize diversity, which can be leveraged for targeted crop improvement. New methods for incorporating biological network information in genomic selection models to predict phenotype from genotype will be explored.

Funding acknowledgement: National Science Foundation (NSF, IOS-1733606)

P232 

## **Reticulon proteins act as autophagic receptors to regulate endoplasmic reticulum (ER) turnover during ER stress in maize aleurone cells**

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The maize aleurone is a single layer of cells that represents the endosperm epidermis. Aleurone cells accumulate large amounts of storage compounds such as proteins, lipids, and over 70% of the endosperm minerals (phosphate, magnesium, potassium, iron, and calcium). In aleurone cells, the endoplasmic reticulum (ER) is central to storage protein and lipid synthesis as well as lipid storage. During the genetic analysis of aleurone development, we discovered three independent mutations in a reticulon gene (*Rtn2*) that drastically suppress the autophagic turnover of ER and other organelles in aleurone cells. Autophagy controls the delivery of cytoplasmic components (including organelles) to the vacuole for degradation. We have found that *Rtn2* localizes to the ER and acts as an autophagy receptor by interacting with ATG8, a key component for the progression of autophagy. Induction of autophagic ER degradation by drug-induced ER stress results in the accumulation of *Rtn2*-decorated cargo encapsulated within cytoplasmic autophagosomes, and *Rtn2*-labelled autophagic bodies inside vacuoles. We propose that *Rtn2* mediates the autophagic turnover of the ER during ER stress and plays a critical role in ER homeostasis within maize aleurone cells.

Funding acknowledgement: National Science Foundation (NSF), University of Wisconsin-Madison.

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## **RNA Binding Motif Protein 48 is required for U12 splicing and maize endosperm differentiation**

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The last eukaryotic common ancestor had two classes of introns that are still found in most eukaryotic lineages. Common U2-type and rare U12-type introns are spliced by the major and minor spliceosomes, respectively. Relatively few splicing factors have been shown to be specific to the minor spliceosome. We found that the maize RNA Binding Motif Protein48 (RBM48) is a U12 splicing factor that functions to promote cell differentiation and repress cell proliferation. RBM48 is coselected with the U12 splicing factor, ZRSR2/RGH3. Protein-protein interactions between RBM48, RGH3, and U2 Auxiliary Factor (U2AF) subunits suggest major and minor spliceosome factors required for intron recognition form complexes with RBM48. Human RBM48 interacts with ARMC7. Maize RBM48 and ARMC7 have a conserved protein-protein interaction. These data predict that RBM48 is likely to function in U12 splicing throughout eukaryotes and that U12 splicing promotes endosperm cell differentiation in maize.

Funding acknowledgement: National Science Foundation (NSF)

P234

### Root pattern formation in response to directional water perception

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Roots have important functions in anchorage, and uptake of water and nutrients. Efficient water and nutrient uptake depend on root system architecture that is determined by branching. Roots respond to environmental cues like gravity, water and nutrient availability to inform their decisions for growth and branching. The direction of root branching can be regulated by spatial differences in moisture availability around the root, a process termed hydropatterning. The maize inbred lines B73 and Oh7B were identified to have significant differences in their hydropatterning behavior. Using Nested Association Mapping (NAM) of the Recombinant Inbred Lines (RILs) from B73 and Oh7B, three Quantitative Trait Loci (QTLs) were identified that are associated with hydropatterning. Current work aims to genetically characterize these loci to determine causal genes involved in hydropatterning and to understand the relevance of hydropatterning on plant performance in the field. Finding those genes may improve our understanding on how plants perceive water in their environment. Preliminary experiments were conducted to analyze the influence of the identified locus on chromosome 2 on hydropatterning. Near-Isogenic Lines (NILs) that are fixed either for the B73 or Oh7B allele at this locus were grown in a field to study differences in root architecture. In parallel, the Goodman diversity panel of distinctive maize lines is being screened for differences in hydropatterning. This will give further insight into variation in the hydropatterning trait across the genetic diversity of maize and enable a Genome Wide Association Study (GWAS) to find additional markers connected with the trait.

Funding acknowledgement: Department of Energy (DOE), Howard Hughes Medical Institute, Simons Foundation

P235

### Sequencing analysis of maize semi-dominant mutants *Suppressor of sessile spikelets 2* and *3* (*Sos2/Sos3*)

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The maize semi-dominant mutants, *Suppressor of sessile spikelets 2* (*Sos2*) and *Suppressor of sessile spikelets 3* (*Sos3*) have been phenotypically characterized to have defects in the developmental progression of meristems that give rise to the male and female reproductive structures; the tassel and the ear respectively. In order to determine the gene responsible for these two phenotypes, leaf tissue was collected from *Sos2* and *Sos3* plants that had been introgressed into a W22 background, then pooled by mutant, sequenced, and mapped to the W22 reference genome. The results indicated seven possible candidate gene regions, four of which were shared between *Sos2* and *Sos3* mutants. This research aims to further narrow down the window in which the candidate gene or genes responsible for these two phenotypes reside. The results suggest that our W22 line differs from the W22 reference genome, and that regions of the genome have been indirectly selected in both *Sos2* and *Sos3* due to shared modifiers. In addition, confocal analysis was performed with *Sos2* and *Sos3* crossed to an RFP fluorescent line that marks WUSCHEL expression. This analysis provides further insight on the effects of the *Sos2* and *Sos3* genes on the stem cell niche in maize reproductive meristems.

Funding acknowledgement: National Science Foundation (NSF)

P236 

### Single-cell RNA-Seq analysis of maize shoot apices

(submitted by Jack Satterlee <[jws429@cornell.edu](mailto:jws429@cornell.edu)>)

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Plant development is dependent on short and long range cell-cell signals which establish a rich informational landscape to direct cellular, tissue, and organ level homeostasis and differentiation. Single-cell genomic technologies offer potentially unprecedented insights into the means by which individual cells coordinate their fate during development. Yet, the high-throughput genetic analysis of single plant cells has remained challenging owing to the need for cell wall digestion and attention to the tissue-specific conditions under which viable protoplasts can be isolated. Here we present a method for single-cell RNA-Seq analysis of cells derived from the maize shoot apex by combining FACS-based cell-viability discrimination with Cel-Seq2, a single-cell RNA-Seq library preparation platform. We classify cells into broad cell types based on existing in situ hybridization and bulk transcriptomic data. Using Cel-Seq2 we are able to capture a far larger proportion of the transcriptome than with higher throughput droplet-based methods. This high transcript capture efficiency of Cel-Seq2 enables the robust partitioning of low abundance cell types, including a small number of cells expressing the ligule marker *liguleless1*. We ultimately aim to use this technique to resolve the cellular heterogeneity within the maize shoot apical meristem (SAM) and leaf primordia to better understand the genetic basis of SAM homeostasis and cell differentiation.

Gene / Gene Models described: *lg1*; GRMZM2G036297

Funding acknowledgement: National Science Foundation (NSF)

P237

### Somatic embryogenesis and transformation of tropical maize inbred lines

(submitted by Andrea Carneiro <[andrea.carneiro@embrapa.br](mailto:andrea.carneiro@embrapa.br)>)

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Somatic embryogenesis is the most used technique for the regeneration of transgenic maize cells. However, the ability to generate somatic embryos is considered genotype-dependent. Most of the transgenic maize plants were produced using the hybrid HiII, which is a genotype highly embryogenic, but displays poor agronomic performance at tropical conditions. In an effort to detect tropical genotypes that associate a high somatic embryogenesis response and good agronomic performance, we evaluated 20 elite inbred lines from the Embrapa Maize and Sorghum Germoplasm collection, Brazil. Immature maize embryos, between 1.2 and 2.0 mm were cultured during 8 weeks in N6 medium supplemented with 1.5, 5.0 and 10 mg/L 2,4-D. The percentage of Type I and Type II somatic embryogenic callus and plant regeneration were recorded in 360 embryos per line. A total of 8 maize lines reached more than 50% calli formation and 70% of these calli regenerated plants after cultivation in MS medium supplemented with 60 g/L sucrose and 1.25 mg/L CuSO<sub>4</sub>. Immature embryos in one inbred line L3 were transformed via *Agrobacterium tumefaciens* harboring the binary vector containing the genes *uidA* and *bar* under the control of the *CaMV35S* promoter. Transgenic calli were formed in the presence of 10 mg/L 2,4-D and 3 mg/L bialaphos at 3.3% efficiency; all calli selected were able to regenerate transgenic plants. Thus, it was selected 8 promising inbred lines as alternative to HiII and it was developed for the L3 inbred tropical line a transformation protocol via *A. tumefaciens*.

Funding acknowledgement: BNDES, FAPEMIG, Embrapa



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## Specialization of the separate male and female inflorescences of maize

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The genetic origin of new cell types, tissues, and organs is a fundamental question of evolutionary and developmental biology. Examples of newly created body parts over the evolution of land plants include vascular tissue, seeds, floral organ, and embryo. But in both plants and animals, the emergence of new specialized body parts is a rare process. One model believes that the emergences of new specialized body parts are constrained by the total number of certain types of transcription factors which are recalcitrant to most types of gene duplication, but not to the whole genome duplication (WGD). The model system *Zea mays* produces two specialized types of inflorescences (tassel and ear) for male and female reproduction which have been shown to be controlled by distinct genetic architectures, while all the other genera in the grass tribe (Andropogoneae) produce only a single type of inflorescence, such as *Sorghum bicolor*. Moreover, a whole genome duplication occurred in *Zea mays* about 5-12 million years ago, but not in the closely related species sorghum. This study seeks to test the link between a whole genome duplication in the maize lineage and the evolution of its developmentally distinct inflorescences. Comparative genomics and reverse genetics will be used to characterize the role duplicate genetic factors played in creating the distinct genetic identities of maize tassels and ears. Candidate genes from sorghum and both maize duplicated genomes have been identified by comparing maize and sorghum inflorescences using multiple expression and quantitative genetic data sets. Inflorescence phenotypes will be analyzed in mutants and CRISPR/Cas9 knockouts of candidate genes of maize and sorghum.

Funding acknowledgement: National Science Foundation (NSF)

P239

## The *Barren inflorescence3* mutant is caused by a tandem duplication of the *ZmWUS1* gene

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Plant stem cells are essential for sustaining growth and development throughout a plant life cycle. These stem cells reside at the growing tip of meristems such as the shoot apical meristem (SAM), where they differentiate to produce new organs and proliferate to maintain a stem cell core. This balance between differentiation and proliferation has to be tightly regulated to maintain all meristems. The underlying molecular mechanism involved in meristem maintenance is the well-characterized CLAVATA-WUSCHEL (CLV-WUS) pathway, and the regulation of *WUS* transcription is central to this pathway. In this study, we report the cloning and functional characterization of the dominant *Barren inflorescence3* (*Bif3*) mutant. *Bif3* displays several inflorescence defects, including barren patches in the central spike of tassels and shortened cobs with very few seeds. These defects are caused by a massive increase in meristem size, curiously not accompanied by an increase in lateral organ formation that is normally observed in various fasciated mutants. Map-based cloning and PCR-based chromosome walking revealed a tandem duplication of a 16 kb-fragment carrying the *ZmWUS1* gene in the *Bif3* mutant. By using targeted EMS mutagenesis and CRISPR-Cas9 knockout approaches, we demonstrate that the duplicated gene is the molecular cause of the *Bif3* phenotype. The tandem duplication causes a peculiar change in *ZmWUS1* pattern of expression, and generates an architectural rearrangement in inflorescence meristems, whereby bilaterally symmetric structures are changed into radially symmetric ones. Through genetic interactions, we find that mutations in genes involved in the negative regulation of *ZmWUS1* all enhance the inflorescence phenotypes of *Bif3*. We are currently investigating the regulatory changes in *ZmWUS1* that generated radially symmetric meristems, and how these meristems respond to different stimuli.

Gene / Gene Models described: *ZmWUS1*; GRMZM2G047448

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P240

## The lateral suppressor1 (*las1*) gene is required for axillary meristem development in maize

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*Zea mays* (maize) develops branches through the initiation and development of axillary meristems. Female reproductive inflorescences, ears, are born on axillary branches. Previously characterized *barren stalk* (*ba*) mutants, *ba1* and *ba2*, fail to produce ear shoots and encode a transcription factor and interacting protein, respectively, that function downstream of auxin. A new mutant, *lateral suppressor1* (*las1*), was identified in an A619 background EMS population as a novel locus controlling axillary meristem development as allelism tests with *ba1* and *ba2* complemented the mutant phenotypes. The *las1* mutant fails to initiate axillary ear branches and the grooves on the internodes that normally bear ears do not develop. Mutants also have shorter primary and fewer secondary branches in the tassel. Double mutant analysis with *teosinte branched1* (*tb1*) and *las1* show that the *las1* gene incompletely suppresses tiller branching, induced by *tb1*, indicating that *las1* plants are still able to form vegetative axillary meristems. To identify the molecular mutation responsible for the *las1* phenotype we used a next generation sequencing and bulk-segregant analysis approach to map the *las1* locus. A SNP was identified within the map window in the coding sequence of a candidate gene that induced a premature stop codon and truncated the coding sequence by 120 nucleotides. This SNP completely co-segregates with the *las1* phenotype. Current endeavors are underway to identify a second allele of *las1* and to determine the placement of *las1* within the pathway regulating ear branch development. These results indicate that *las1* functions in vegetative and reproductive axillary meristem formation in maize.

Funding acknowledgement: National Science Foundation (NSF)

P241

## The maize heterotrimeric G-protein $\beta$ subunit functions in shoot meristem regulation and immune response.

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The ear kernel row number (KRN) is an important agronomic trait and correlated with the inflorescence meristem size. We have shown that the maize *COMPACT PLANT2* (*CT2*) gene, which encodes the  $\alpha$  subunit of heterotrimeric G-protein complex ( $G\alpha$ ), functions in the CLAVATA (*CLV*) pathway to control meristem size and KRN through its interaction with FASCIATED EAR 2 (*FEA2*), a homolog of *CLV2*. The heterotrimeric G protein complex consists of  $G\alpha$ ,  $G\beta$  and  $G\gamma$  subunits. To understand the functions of other G-protein subunits, we used CRISPR-Cas9 to knockout the  $G\beta$ -subunit gene, *ZmGB1* and found that *Zmgb1* mutants were lethal at the seedling stage. The *Zmgb1* mutants showed cell death, over-accumulation of H<sub>2</sub>O<sub>2</sub> and salicylic acid, constitutive activation of MAP-kinases, and up-regulation of *PR1* (*PATHOGENESIS-RELATED 1*) and *PR5*, two immune marker genes, suggesting that the lethality of *Zmgb1* mutants is caused by autoimmunity. We crossed *Zmgb1* (using viable heterozygotes) to the diverse maize NAM founder lines, and found that *Zmgb1* lethality can be suppressed in the CML103 background. The lethality suppressed *Zmgb1* plants have larger SAMs and fasciated ears, suggesting that *ZmGB1* functions in inflorescence meristem development, like  $G\alpha$ . We mapped the suppressor of *Zmgb1* lethality in CML103, and identified an R gene as a candidate, suggesting it acts as a 'guardee' in maize, unlike in Arabidopsis where  $G\beta$  mutants are viable. Our study demonstrates that maize heterotrimeric G-proteins function in controlling meristem size during ear development, as well as in crosstalk with immune responses. Therefore, tweaking G protein signaling has the potential to optimize the tradeoff between yield and disease resistance.

Gene / Gene Models described: *ct2*, *Zmgb1*; Zm00001d027886, Zm00001d033422

Funding acknowledgement: United States Department of Agriculture (USDA, grant no. 2018-67013-27420)

P242

## The maize NIN-LIKE PROTEIN 5 (ZmNLP5) transcription factor regulates nitrogen response in maize

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Maize is an important crop that exhibits marked growth and yield response to supplemental nitrogen (N). However, the molecular mechanism underlying maize's response to N is still not fully understood. Here, we report functional characterization of a maize NIN-LIKE PROTEIN ZmNLP5, which has been discovered as a central hub in a molecular network regulating N signaling and metabolism. Predominantly expressed in roots (especially root tips) and vascular tissues, ZmNLP5 was shown to rapidly respond to nitrate treatment. We showed that when compared with WT seedlings, the *zmnlp5* mutant seedlings accumulated less nitrate, nitrite in the root tissues and ammonium in the shoot tissues under N deficiency. In the *zmnlp5* mutant plants, loss of ZmNLP5 function resulted in changes of expression for a significant number of genes involved in N signaling and metabolism, including the maize nitrite reductase ZmNIR1.1, which reduces nitrite to ammonium in the N assimilation pathway. We further show that ZmNLP5 directly regulates expression of ZmNIR1.1 by binding to the nitrate-responsive cis-element (NRE) at 5'UTR of the gene, and that the mutation in ZmNLP5 in the mutant plants suppressed expression of *ZmNIR1.1* and led to relative high nitrite abundance in root tips and shoot tissues of the *zmnlp5* mutant seedlings grown under N limitation in comparing with WT seedlings. Besides, during the mature stage, we found that the *zmnlp5* mutant plants accumulate less nitrogen than WT plants in the ear leaves and seed kernels under N deficiency. Interestingly, a natural loss-of-function allele of ZmNLP5 was identified in a maize breeding line Mo17, which confers the less N accumulation in the ear leaves resembling that of the *zmnlp5* mutant plants. Taken together, our findings provide evidence that ZmNLP5 is a key regulator mediating plant response to N in maize.

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P243

**The maize pollen-enriched gene *LARP6C1*, a member of the La-domain family of RNA-binding proteins, contributes to pollen competitive ability**

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Members of the La-Related Protein family (LARPs) contain a conserved La motif, which has been associated with RNA-binding activity. Expression of the maize gene GRMZM2G323499/Zm00001d018613, a member of the LARP family, is highly specific to pollen, based on both transcriptomic and proteomic assays. This suggests a pollen-specific RNA regulatory function for the protein, designated *LARP6C1* based on a high degree of sequence similarity to the LARP6 subfamily in *Arabidopsis*. To test this hypothesis, a *DsGFP* transposable element insertion in the gene was obtained from the Dooner/Du collection (Li et al. 2013). Sequencing confirmed that the *R82C05* insertion is in an exon, and thus likely interferes with *LARP6C1* function. Tracking inheritance of the insertion via its endosperm-expressed GFP indicated that the mutation was associated with reduced transmission from a heterozygous plant when crossed as a male (ranging from 6.88% to 26.55%), but not as a female. Furthermore, this transmission defect was significantly relieved (ranging from 29.72% to 100%) when less pollen was applied to the silk, presumably reducing competition between mutant and wildtype pollen. Initial in vitro experiments indicated that the mutant pollen germinated at a significant lower rate than wildtype pollen. However, pollen grain diameter and nuclei count showed no differences between wildtype and mutant pollen. These results are consistent with the hypothesis, supporting the idea that *LARP6C1* provides an important male-specific function in the haploid gametophyte during the highly competitive phase of pollen germination and/or pollen tube growth. Partially supported by grant IOS-1340050 from the US NSF to JEF, and 201806995022 from the CSC to Lian Zhou.

Gene / Gene Models described: *LARP6C1*; GRMZM2G323499/Zm00001d018613

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

P244 

## The OPAQUE1/DISCORDIA2 myosin XI is required for asymmetric cell division

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During development, asymmetric cell divisions contribute to cellular patterning by determining the relative placement of various cell types. Cell fate decisions are often tied to asymmetric divisions. Stereotypic divisions that form maize stomata have been a model for studying asymmetric division. Monocot grass stomata are made of 4 cells: 2 guard cells flanked by a pair of subsidiary cells. The asymmetric division of a subsidiary mother cell yields a subsidiary cell and a pavement cell, and is a useful cellular model for determining the molecular determinants that orchestrate asymmetric divisions. Asymmetric division occurs in three phases: (I) cell polarization, (II) division plane establishment and maintenance and (III) mitosis & cytokinesis. Actin networks play a role in each of these three phases. We hypothesized the class XI myosin, OPAQUE1 (O1), would play a role in asymmetric division during stomatal development. Indeed, *o1* mutants show stomatal patterning defects caused by aberrant subsidiary mother cell asymmetric divisions. However, nuclear polarization is normal in *o1*, indicating a role for O1 post-polarization. *Discordia2*, previously shown to be important for asymmetric division, is an allele of *O1*. Localization data indicate O1 associates with cytoskeletal structures and analysis of cytoskeletal elements in *o1* mutants confirms a role for O1 post-polarization. Live cell-imaging indicates that *o1* have a phragmoplast guidance defect. To determine how O1 might guide phragmoplast expansion towards the division site, we determined which proteins interact with O1. Co-IP/MS data indicate that O1 interacts a variety of cytoskeletal proteins, including maize orthologs of the Arabidopsis protein PHRAGMOPLAST ORIENTING KINESIN1/2 (POK1/2). Physical interaction between the microtubule motor POK1/2 and the actin motor O1 tie together these two cytoskeletons.

Gene / Gene Models described: *o1*; Zm00001d052110

Funding acknowledgement: National Science Foundation (NSF)

P245

## The role of auxin in vein patterning in maize leaves

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The plant hormone, auxin, plays a central role in vein formation and patterning in leaves. The widely accepted hypothesis for the control of vein patterning, ‘auxin canalization’, involves convergent and polar auxin transport (PAT). This mechanism is well studied in dicots, particularly in Arabidopsis. Studies using PAT markers have demonstrated that the same mechanism controls early vein patterning in grasses. However, how PAT controls the vein patterning during later stages of grass leaf expansion has not been sufficiently explored. Vein patterning in grasses is different from that of a typical dicot as longitudinal minor veins originate from the tip and extend toward the base of the leaf, running in parallel with already-established veins. The density of veins varies among grass species, with C4 species, like maize, having denser and narrowly spaced veins. Recently, it has been shown that local auxin biosynthesis plays a critical role in vein patterning, in addition to PAT. In the present study, we observed that transitional and adult leaves in the auxin-deficient maize mutant, *vanishing tassel2* (*vt2*), have a significantly higher number of intervening veins (veins in between lateral veins) with no obvious defects in the leaf anatomy. In addition, *vt2* mutants have an irregular frequency distribution of intervening veins compared to normal. These phenotypes indicate that, apart from PAT, vein patterning in maize is controlled by mechanisms dependent on auxin biosynthesis. We hypothesize that the low auxin condition in *vt2* is either misactivating a signaling program for intervening vein development, or modifying signals that regulate developmental transitions during leaf expansion.

P246

## The transcriptional corepressor REL2 interacts with IDS1 and SID1 to regulate spikelet and floret development in maize

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Maize florets are organized into units called spikelets, resulting from the activity of spikelet meristems, and ultimately consist of two florets enclosed by a pair of sterile bracts called glumes. The spikelet meristem is determinate, producing one floral meristem and then converting into a second floral meristem. The AP2-type transcription factor IDS1 was originally identified as a regulator of spikelet meristem determinacy. A paralog of *IDS1*, *SISTER OF INDETERMINATE SPIKELET1 (SID1)*, is also present in maize. Although single *sid1* mutants have no visible phenotype, when combined with *ids1*, the fate of several meristems in the inflorescences is affected. Fewer rows of axillary meristems form in both ear and tassel, spikelet meristems are indeterminate and the normal patterning of floral organ initiation is disrupted. Yeast-2 hybrid screen of a maize inflorescence library conducted in our lab using the transcriptional corepressor REL2 as a bait identified both IDS1 and SID1 as interacting proteins. This suggests that *IDS1* and *SID1* play a role in repressing meristem indeterminacy and in the patterning of floral organs, possibly by directly engaging REL2 co-repressors to repress transcription of downstream targets. Our molecular interaction data show that IDS1 and SID1 recruit REL2 by the well-known EAR repressor motif. In agreement with our molecular interaction data, genetic analysis indicates that *rel2* enhances the indeterminate phenotypes of *ids1* mutants and *ids1; sid1* double mutants, suggesting that additional transcription factors function in repressing meristem indeterminacy. Furthermore, first and second whorl floral organs show partial homeotic transformation in silk-like structures in both ear and tassel of *ids1; rel2* double mutants and *ids1; sid1; rel2* triple mutants. This suggests that C-function genes have expanded into the first and second whorls of florets and indicates that REL2 works with IDS1 and SID1 to regulate both meristem determinacy and floral organ identity.

Gene / Gene Models described: *rel2*, *ids1*, *sid1*; GRMZM2G042992, GRMZM5G862109, GRMZM2G176175

Funding acknowledgement: National Science Foundation (NSF)

P247 

## Towards robust transient transformation methods for maize pollen: Manipulating pollen tube germination and growth *in vitro*

(submitted by John Fowler <[fowlerj@science.oregonstate.edu](mailto:fowlerj@science.oregonstate.edu)>)

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Transient transformation methodologies for pollen in tobacco and lily have been useful for experimental investigation of pollen tube development and cell biology. However, no robust methods for similar approaches are currently available in maize. In part, this is likely due to the fragility of maize pollen and pollen tubes in liquid culture and the subsequent reduction of viability following experimental manipulation. We are attempting to develop such methods via a stepwise process. First, by better characterizing pollen tube germination and growth in culture through microscopy. Second, by using defined characteristics to help understand how experimental manipulation reduces pollen tube viability, and by developing methods to mitigate reduced viability where possible. Third, by adapting published protocols to conditions that best preserve maize pollen viability in culture. In liquid culture, maize pollen hydrates and the vegetative cell subsequently expands through the single pore within the first few minutes after exposure to media, followed by initiation of pollen tube growth proper. Sensitivity to physical manipulation increases dramatically during this phase of pollen tube growth. However, suspension of pollen in paraffin oil delays this increased sensitivity, and thus appears to be a potential medium for implementing a transformation protocol. Attempts at reproducing a pollen magnetofection approach to transient transformation showed no success. Results using more traditional approaches (e.g., biolistics) will be presented.

Funding acknowledgement: National Science Foundation (NSF), OSU College of Agricultural Sciences, China Scholarship Council



P248 

## **Transcriptional networks regulating maize inflorescence development and their perturbation in response to early season drought stress**

(submitted by Edoardo Bertolini <[ebertolini@danforthcenter.org](mailto:ebertolini@danforthcenter.org)>)

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Drought stress can have devastating effects on maize productivity. While numerous studies have focused on maize response to drought during the critical anthesis-silking interval or during grain set and fill, relatively little attention has been given to the potential effects of early season drought on development of the ear and tassel. An important component of yield potential is inflorescence architecture, which is largely determined very early in development, long before emergence of the mature structures. Here, we investigate the effects of early season drought on ear and tassel development at the transcriptional level and determine points of intersect between endogenous developmental pathways and stress response signals. We integrated numerous RNA-seq datasets based on specific stages of early maize ear and tassel development and in various mutant backgrounds to construct a comprehensive co-expression network map as a baseline for normal inflorescence development. We then overlaid expression profiles generated from developing ear and tassel primordia in response to drought to determine the extent of network rewiring. The drought data were generated from a controlled, greenhouse-based experiment where young maize plants were either subject to water withholding or used as controls, and inflorescence meristem samples collected over a time course. Multiple independent drought experiments were conducted, which allowed us to sample different meristem types from inflorescence primordia and gain more insight into spatiotemporal responses to stress. We observed perturbations in transcription of metabolic pathway components, notably sugar metabolism and GA biosynthesis and signaling. We are further investigating rewiring of hub transcription factor hierarchies in response to drought. Together, these analyses provide insight into how developmental networks interface with abiotic stress response pathways in control of inflorescence architecture. This work acknowledges funding from NSF-PGRP.

P249 

## **Transcriptomic dissection of the spatial-temporal gene expression patterns of maize silks: Implications for development, defense, and reproduction**

(submitted by Colton McNinch <[cmcninch@iastate.edu](mailto:cmcninch@iastate.edu)>)

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Maize silks are a highly dynamic, fast growing tissue. Silk growth is dependent on both cell division and elongation along the lengths of the silks, with cell division ceasing from the silk tip to base. This developmental gradient is overlaid with the transition of silks from the encasing husk-leaves into the external, often harsh environment. Meanwhile, a spatial gradient of various communication signals exist to allow the recognition and germination of compatible pollen grains, and to properly guide their growth toward the ovule. As such, we predict that complex spatial-temporal patterns of gene expression exist to promote proper growth and protection of this tissue and to enhance its receptivity toward pollen. To explore the dynamics of the gene networks expressed along the silk length, RNA-Seq was conducted on five contiguous regions (three husk-encased and two emerged) of unpollinated silks that were harvested 3-days post-silk emergence from inbred lines B73 and Mo17 in each of two growing seasons. After read alignment and quantification using the B73 and Mo17 reference genomes, several key findings have emerged: (1) the most dramatic changes in gene expression occur between neighboring husk-encased and emerged tissues; (2) a similar number of genes are differentially expressed between encased and emerged tissues in the two inbred lines and the functionality of these genes are enriched for stress-related and cell-to-cell signaling responses; (3) growing season has a strong yet unequal influence on gene expression between the inbred lines; and (4) co-expression analyses on the two inbred lines have revealed conserved differential expression of genes in the phenylpropanoid and cysteine-rich peptide production pathways, which are essential for defense, with cysteine-rich peptides also playing important roles in cell-to-cell signaling that could govern recognition and receptivity of pollen.

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

P250

## **Using mutants to identify new branching loci in *Setaria italica* (foxtail millet)**

(submitted by Yisel Carrillo Tarazona <[yiselja@okstate.edu](mailto:yiselja@okstate.edu)>)

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In economically important crops, plant architecture greatly affects crop yield. Tillers (basal branches) are an important architectural feature in the grass family, yet the genetic and hormonal pathways controlling tiller production in grasses are imperfectly understood. The two best-studied species are rice and maize, but these two have significantly different architectures and differ also in what we know of their genetic regulation. We propose to use *Setaria italica* (foxtail millet), in the tribe Paniceae (sister to the Andropogoneae, in which maize is found) as a model plant to expand the knowledge of genes and hormones involved in the production of tillers in the grasses. We have initiated a study of tiller mutants in *Setaria italica*, and are focusing on one mutant that produces an abundance of secondary and tertiary tillers when compared to wild-type. Bulk-segregant analysis of an F2 population obtained from the backcross between foxtail millet and mutant was performed, in order to identify SNPs responsible for the mutant phenotype. We present both an analysis of the development of the mutant phenotype as well as the results of our BSA analysis.

Funding acknowledgement: National Science Foundation (NSF)

P251

## **Where is the transgene? A method to identify genomic locations for transgenes in maize.**

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The use of transgenic plants has become a standard approach to study a host of biological processes in maize including, for example, gene function, hormone signaling, genomic rearrangements, transposon biology, and in genome editing technologies. Identification of genomic insertion sites for transgenes is important in the production of insertional mutagenesis collections, and for entry into the de-regulatory pipeline for a value-added transgene. Moreover, as we learn more about the impact genomic context can have on gene expression, it becomes desirable to know the location of a transgene because it may impact the interpretation of gene function. Approaches using iPCR and tail-PCR have been used successfully to accomplish this, however these protocols are typically performed on individual line(s) in a low through-put fashion. Short-read sequencing efforts have been successfully applied to map transgenes in species with smaller, less complex genomes such as *Arabidopsis* and *Brachypodium*. We wanted to determine if long-read (PacBio) sequencing technology could be efficiently used to accurately map a collection of transgenic events in maize, where the genome is large and complex. We developed a streptavidin-based enrichment protocol to generate a single library of barcoded flanks from 20 single-locus transgenic maize lines. A bioinformatics pipeline was designed to analyze the data and successfully place the flanks on the maize genome. Unique locations were identified for 16 of the 20 lines. Computer simulations were performed with sequences from the insertion locations to evaluate whether placement accuracy benefited from the use of longer reads.

Funding acknowledgement: National Science Foundation (NSF)

P252

## **De novo centromere formation on a chromosome fragment with an inactive centromere**

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A functional centromere, which is cytologically identified as the major chromosomal constriction, is responsible for accurate chromosome orientation and segregation in cell division. In maize, as in most eukaryotes, centromere activity is determined epigenetically via the presence of centromeric histone H3 variant CENH3. Previous work had identified 9-Bic-1 (9-B inactivated centromere-1), which is an epigenetically silenced B centromere that was translocated to the short arm of chromosome 9. This chromosome is stable in isolation but when normal B chromosomes are added to the genotype, it will attempt to undergo nondisjunction at the second pollen mitosis and usually fracture the chromosome in 9S. These broken chromosomes allow a test of whether the inactive centromere is reactivated or whether a de novo centromere is formed elsewhere on the chromosome to allow recovery of fragments. Ten minichromosome fragments were recovered and are in various stages of analysis. For one of them, breakpoint determination by sequencing showed that minichromosome 1104 had the same breakpoint as 9-Bic-1 in the B centromere region, which indicates it is derived from 9-Bic-1. To test whether de novo centromere formation or reactivation of the B centromere occurred, we performed genome-wide mapping of CENH3 binding sites and found a 500-kb de novo centromere on the short arm of chromosome 9 within the limits of the 9S portion of the minichromosome. Our results indicate that reactivation of inactive centromeric sequences was not favored relative to de novo centromere formation over unique sequences in this case.

Gene / Gene Models described: *c1*; Zm00001d044975

Funding acknowledgement: National Science Foundation (NSF)

**P253**

**Analysis of organellar DNA sequences in the B-chromosome of maize**

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Maize nuclear genomes contain insertions of both mitochondrial and chloroplast DNA – referred to as nuclear mitochondrial sequences (NUMTs) and nuclear plastid sequences (NUPTs), respectively. Using fluorescent in situ hybridization (FISH), we have demonstrated that the supernumerary B-chromosome of maize also contains large insertions of mitochondrial DNA. The exact origin of the B-chromosome is uncertain; however, previous research has determined its structure to be mostly heterochromatic, containing a collection of repetitive sequences. Currently, we are using a draft B-chromosome sequence produced by an international consortium to compare the B-chromosome NUMTs to the mitochondrial genotypes of maize and its relatives. Identifying the origins of the B-chromosome NUMTs may provide insight into whether they are from a recent contributor or from a more distant evolutionary source.

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**P254**

**Centromere birth and death after pollen irradiation**

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B chromosomes are supernumerary chromosomes, which provide a model for the study of centromere function because the chromosome is nonvital. Here we studied the process of centromere formation, centromere activity and chromosomal fusion following pollen irradiation in maize with multiple B chromosomes whose fracture would not be consequential to survival. The pollen from plants with B chromosomes was gamma irradiated and then applied to normal maize ears without B chromosomes. Among ~8000 first generation seedlings, we recognized many B-A translocations. Centromere expansions and ring chromosomes were also observed. Importantly, chromosomal fragments were found without canonical centromere sequences. Two example cases were examined via CENH3 ChIP-Seq and revealed the presence of de novo centromere formation over unique sequences. Many structural dicentric chromosomes were also observed but a substantial fraction of these show only a single primary constriction suggesting centromere inactivation of one of the pair. In two example cases, immunolabeling for H2A-pThr133, a biochemical marker of centromere activity, illustrated the inactivation of one of the two centromeres. These results illustrate the regular occurrence of both centromere birth and death following chromosomal rearrangement and that these events occur during a narrow developmental windows spanning one to potentially only a few cell cycles in order for the rearranged chromosomes to be recovered.

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P255

### **Characterization of acentrosomal meiotic spindle formation in *Zea mays***

(submitted by Sarah Stinebaugh <[ssineba@hamilton.edu](mailto:ssineba@hamilton.edu)>)

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Reproduction relies on a cell's ability to properly segregate genetic material into haploid gametes. Spindles divide genetic material in a cell, and are therefore responsible for ensuring that proper chromosome segregation occurs during meiosis. In the absence of a centrosome, a microtubule organizing center (MTOC), dividing plant cells must use a method of "self-organization." While acentrosomal plant meiosis has been observed using fixed images, observing this process in live cells can offer a perspective on meiotic spindle formation dynamics that otherwise cannot be seen. We observed meiosis in male maize cells, visualized through live fluorescence microscopy techniques using CFP-tubulin and SYTO12-stained chromosomes. We characterized rate of spindle assembly and disassembly, frequency of errors and corrections, focusing of spindle poles and formation of the phragmoplast. Developing a baseline model of wild-type meiotic spindle dynamics will allow us to investigate pathways responsible for self-organization and understand errors that occur in this process.

P256

### **DNA Methylation and gene expression comparison between maize inbred lines with highly different mean chiasmata numbers**

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Chiasmata are cytological manifestations of meiotic crossing over between homologous chromosomes and are one of the possible outcomes of DNA double strand break (DSB) repair. In animals and yeast, the frequency of crossing over remains stable despite large variations in DSB numbers, a concept known as crossover homeostasis. In maize however, there is a strong correlation between DSB and crossover frequencies, indicating that homeostasis mechanisms only play a minor role during meiosis. In an effort to understand these differences, we compared DNA methylation patterns of isolated meiotic cells and anthers undergoing meiosis between an inbred line with a low DSB/chiasmata frequency (CML228) to inbred lines with typical DSB/chiasmata frequencies (B73 and Mo17). Results of whole genome bisulfite sequencing revealed that there are no general DNA methylation pattern differences in meiocytes and anthers between the low and high DSB/chiasmata inbred lines in all sequence contexts. In contrast to the DNA methylation result, many differentially expressed genes were detected in an RNA-Seq study comparing these inbred lines. The differentially expressed meiosis-related genes include those that are known for their role in class I (interference sensitive) crossovers. Among them, *Atm* – a gene expressing a protein kinase known to be involved in DSB and crossover control – is consistently expressed higher in CML228 compared to both B73 and Mo17. This is consistent with *Atm*'s proposed inhibitory role in DSB and crossover formation previously observed in mice and yeast. In summary, our findings indicate DNA methylation may not be a key factor in determining DSB/chiasmata frequencies in maize. It also suggests a conserved role for *Atm* in inhibiting the formation of DSBs, and hence crossovers in maize.

Gene / Gene Models described: Zm00001d040166

Funding acknowledgement: National Science Foundation (NSF)

P257

## **Evidence that the B specific centromere sequence is the target for nondisjunction of the B chromosome**

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The B chromosome has an unusual behavior in that it undergoes nondisjunction (NDJ) at the second pollen mitosis as part of its accumulation mechanism. The centromere undergoes NDJ at this mitosis that produces the two maize sperm and requires the presence of other trans-acting parts of the B chromosome for this to occur, most notably the very terminal region of the long arm. Previous studies indicated that the B chromosome centromere contains CentC satellite and CRM retrotransposons as do the centromeres of the A chromosomes but it also contains a B specific repeat sequence located in and around the functional B centromere. We tested the hypothesis that the B centromere specific repeat is the target for the nondisjunction property of the B centromere. Two B-repeat units, B4a (0.9 kb) and K5 (1.6 kb), were ligated for producing multimer conglomerates of these units, and then co-bombarded into Hi II calli with an Ubiquitin promoted bar selection cassette. Forty three transformation events have been obtained. Fluorescence in situ hybridization for the B repeat array transgene was used to localize the insertions to chromosome arm. One insertion on chromosome arm 5S was crossed to full color W22 with five to eight B chromosomes to supply the trans-acting function. When these heterozygotes for the insertion were crossed as male parents to the a2; R-scm2 tester stock, kernels with pigment mosaicism in the endosperm were observed, consistent with chromosomal breakage in 5S that might result from attempted nondisjunction of an internal insertion. No such phenomenon is observed when either W22 with B chromosomes without the 5S insertion or Hi II with the 5S insertion without B chromosomes were crossed as male parents to the a2; R-scm2 tester stock as controls. While the 5S multimeric array is much smaller than the span of the repeat on the B chromosome, these results are consistent with the proposition that the B centromere specific repeat is the target for producing NDJ.

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Funding acknowledgement: National Science Foundation (NSF)

P258

## **Frequent spindle assembly errors require structural rearrangement to complete meiosis in Zea mays**

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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. The machinery used to pull chromosomes apart is the spindle, a bipolar, microtubule-based structure. Male maize meiocytes lack many of the features that govern the assembly, organization and positioning of the spindle, so we investigated the dynamics of the spindle assembly process in wild-type meiotic cells using live fluorescence microscopy. Cells frequently formed tripolar or multipolar spindles; approximately half of all assembly events initially formed a tripolar spindle. Tripolar spindles were re-organized into correctly shaped bipolar spindles during prometaphase chromosome congression. We probed spindle assembly by inhibiting two known acentrosomal pathways and found evidence that both the RanGTP and Chromosome Passenger Complex pathways maybe critical for directly assembly. When components of either of these pathways were inhibited, meiotic progression arrested at metaphase. The observed correction spindle structure before anaphase and the induced metaphase arrest suggest an active and essential role for the spindle checkpoint in maize meiosis. In other systems, the spindle checkpoint monitors the kinetochore, the protein interface between chromosomes and the spindle. We investigated the structure of maize kinetochores in meiotic metaphase using electron microscopy, and found a maximum of 18 attached microtubules. tension or attachment of microtubules to the kinetochore. The frequency of substantial errors in assembly and their subsequent correction before anaphase suggests an active and essential role for the spindle assembly checkpoint in the progression of meiosis.



P259

## Identification and characterization of Haspin kinase as an essential kinase during cell division in maize

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Haspin (haploid germ cell-specific nuclear protein kinase) is member of a divergent group of the eukaryotic protein kinase family that is conserved in many species including fungi, plants and animals. RNAi-mediated depletion of Haspin led to a reduced level of H3T3ph and disrupted sister chromatid association, while overexpression of Haspin resulted in an increased level of H3T3ph and hindered cohesion release. Though Haspin has been reported for phosphorylating H3 at Thr3 and for a developmental function in Arabidopsis, its precise role in regulating cell division has not been investigated in plants. Here, we characterized maize Haspin (ZmHaspin), responsible for H3T3ph and determined its localization in nucleus. We used CRISPR/Cas9 genome-editing technology to knock out ZmHaspin for gene function validation. The transgenic seedlings with higher mutation ratio show a slow growth phenotype and cannot survive after two weeks. At the level of chromosomes, the ZmHaspin mutants exhibited abnormal alignment, which led to subsequent improper chromosome segregation with the presence of lagging chromosomes in anaphase. As a consequence, micronucleus were detected in interphase. The ZmHaspin mutants also exhibited abnormal spindle morphology including spindle polarity and spindle microtubule organization. Taken together, these results indicate that Haspin is required for bivalent alignment and normal spindle organization to guarantee accurate chromosome segregation in maize.

Gene / Gene Models described: *Haspin*; Zm00001d006938

Funding acknowledgement: National Science Foundation of China (NSFC)

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## Kn11 displays distinct architecture with spindle assembly checkpoint and is essential for chromosome segregation in maize

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The Kn11-Mis12-Ndc80 (KMN) network is an essential component of the kinetochore-microtubule attachment (KT-MT) interface, which is required for faithful chromosome segregation and genomic stability of all organisms. However, detecting the homolog of Kn11 protein in plants was difficult, due to a complicated evolutionary history. Here, using the yeast two-hybrid system, we identified the Kn11 candidate in maize, which lost many conserved motifs and displays extensive divergence during evolution. We determined a conserved binding pattern for plant Kn11 and spindle assembly checkpoint (SAC) components, which was not involved with the phosphorylated MELT repeats that are essential for SAC recruitment in mammalian cells and yeast. Immunoassay of ZmKn11 reveals that it localizes to the kinetochore with continuous signal strength during the entire cell cycle, similarly to the signals of Mis12 and Ndc80, indicating that Kn11 is a constitutive feature of the central kinetochore. The functional analysis using uniform mutator and the CRISPR/Cas9 system both indicate ZmKn11 is essential for proper chromosome congression and segregation. Furthermore, the deficiency of ZmKn11 can lead to perturbed chromosome segregation in the cell cycle of embryo and endosperm, which subsequently causes a series of stress responses and corresponding metabolic reactions, ultimately displaying a strong defective seed development. This work sheds light on the mechanism of the conserved KMN network in maize for chromosome congression during cell division.

Gene / Gene Models described: *kn11 Mis12 Ndc80*; Zm00001d046545

Funding acknowledgement: National Natural Science Foundation of China

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## Maize by monet: Demonstrating the utility of whole chromosome oligo-FISH paints

(submitted by Patrice Albert <[albertp@missouri.edu](mailto:albertp@missouri.edu)>)

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Whole chromosome paints that fluorescently label each pair of the ten maize chromosomes were developed using bioinformatics-based selection of unique oligonucleotide sequences that were mass synthesized as PCR-amplifiable, double-stranded DNA libraries ([doi.org/10.1073/pnas.1813957116](https://doi.org/10.1073/pnas.1813957116)). The fluorescent single-stranded cDNA probes were subsequently made via reverse transcription using an intermediary RNA template. Here we demonstrate how the paints can be used, with or without the addition of repeat-based probes, in somatic root tip cells in metaphase or interphase, and in the pachytene stage of meiosis to answer any number of questions. Chromosomal aberrations ranging from simple reciprocal translocations and insertions to complex, multi-chromosome stacked rearrangements, as illustrated with a quadruple B-A translocation, have been clearly visualized. The paints can also be used to identify and track specific chromosomal regions during strain construction or to aid in genotyping species hybrids, for example, those of maize-Tripsacum with different numbers of genomes. In interphase nuclei, the paints can be used to identify the discrete, though diffuse, territories occupied by individual homologues. The ability to follow individual chromosomes during meiosis will be an invaluable tool for the study of chromosomal behavior. Although developed using B73 reference sequences, the oligonucleotide FISH probes selectively paint their target chromosomes on all maize lines tested. Additionally, within the limits of FISH resolution, none of the A chromosome paints hybridize to the maize B chromosome.

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## The condensin subunit SMC2 interacts with SMC4 to compact mitotic chromosome and maintain normal chromosome segregation

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Structural Maintenance of Chromosomes 2 (SMC2) is associated with Structural Maintenance of Chromosomes 4 (SMC4) to form the core of the condensin complexes, which are multi-subunit protein complexes playing a critical role in chromosome assembly and segregation during cell division. Because of the crucial biological function of both proteins, much work has been done about both proteins in various vertebrates, but how they affect cell divisions in plants is still largely unknown. Here, we cloned the cDNA sequences of both genes and showed the dynamic changes of ZmSMC2 and ZmSMC4 during mitosis in maize. ZmSMC2 is associated with ZmSMC4 only by their hinge domains to form a complex. We find that the overexpression of ZmSMC2 cannot improve the condensation degree of mitotic chromosomes perhaps because not enough ZmSMC4 interacts with ZmSMC2 to form the complex controlling chromosome condensation. Cytological analysis of knockdown and knockout transgenic plants of both genes reveals that the deficiency of ZmSMC2 and ZmSMC4 results in aberrant chromosome architecture with increased volume and surface area and abnormal sister chromatid separation. Furthermore, we found that the reduction and deletion of ZmSMC2 and ZmSMC4 results in mislocalized H3S10ph and malformed spindles. Together, these discoveries provide evidence that ZmSMC2 interacts with ZmSMC4 to maintain normal chromosome architecture and accurate segregation in mitosis. The deletion of both proteins affects the location of spindles and results in abnormal chromosome behavior.

Gene / Gene Models described: *ZmSMC2*, *ZmSMC4*; GRMZM2G006452, GRMZM2G383623

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## The deposition of CENH3 is stringently regulated in Maize

(submitted by Jing Yuan <[yuanj@genetics.ac.cn](mailto:yuanj@genetics.ac.cn)>)

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The centromere, as an essential element to control chromosome segregation, is epigenetically determined by CENH3-containing nucleosomes as a functional marker, therefore the accurate deposition of CENH3 is crucial to chromosome transmission. We characterized the deposition of CENH3 in maize by over-expression and mutational analysis. Our results revealed that over-expressing CENH3 in callus is lethal while over-expressing GFP-CENH3 and CENH3-YFP in callus and plants is not and can be partly deposited normally. Different mutations of GFP-CENH3 demonstrated that the Thr in the N terminus was needed for the deposition as a positive phosphorylation site and the last five amino acids in the C terminus are necessary for deposition. Taken together, multiple amino acids or motifs were shown to play essential roles in CENH3 deposition, which is suggested to be stringently regulated in maize.

Funding acknowledgement: National Key Research and Development Program of China, State Key Program of National Natural Science Foundation of China

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## DIVAS Project image processing interventions improve the perceived computational ability of students in the natural sciences and their interest in pursuing computational careers

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The DIVAS (Digital Imaging and Vision Applications in Science) Project aims to build the computational self-efficacy, inform careers, and support the computational thinking skill of undergraduates in the natural sciences using image data as a 'hook'. This NSF-funded project is led by faculty at both Doane University in Crete, Nebraska and St. Edward's University in Austin, TX who represent biology, chemistry, and computer science disciplines. The project has tested a series of curricular and co-curricular image-analysis based interventions starting the first year of college. Program elements include two professional development seminars, image processing modules implemented in chemistry and biology courses, a week-long summer coding workshop that includes basic bash commands, version control using git and basic image processing using Python/OpenCV, and summer research including both pair programming of common problems and independent projects with weekly code reviews. Six first year 'DIVA scholars' were selected each of the first two years of the project. An additional 12 undergraduate students and 12 participants at the graduate-level and above completed the coding workshop and 61 have completed two-day image-processing modules in the classroom. Significant positive effects on student self-efficacy toward computing were observed after the first seminar and coding workshop both years and in both modules in year one. Career path was positively affected by both modules both years and after the first seminar and pair programming in year two. To assess computational thinking (CT), a rubric was designed based off the RADIS framework (Recognize / Analyze / Design / Implement / Support) of code development. This rubric was utilized to measure CT skills before the seminar using written responses to a CT-related problem and after workshops and research using code students developed.

Funding acknowledgement: National Science Foundation (NSF)

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## Evaluating community curation approaches for improving annotation on classical maize gene models

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The classical maize gene families have been very well studied by the maize genetics community. These genes are a gold standard for maize genetics as information is widely available and the majority have been cloned from cDNA. In 2017, we held the first genomic annotation jamboree for the reference Maize B73 (B73RefGen\_V4), using a newly-developed strategy to involve students in the improvement of maize gene models. 419 unique genes in 308 distinct families were evaluated by two new approaches: 1) gene tree visualization alignment tool, and 2) MAKER-P quality metrics. A set of 10-20 classical genes was scored by three students weekly using Gramene's gene tree visualization alignment tool until the list of 419 genes was covered. Once all rounds were completed, the genes flagged were reviewed and the results scored. A total of 72 genes flagged by 2 of 3 curators were deemed "suspicious" and were lined up for analysis with the Apollo genome annotation tool. Of those, 26 genes were excluded due to complex splice patterns (> 4 transcripts) and one failed visual analysis in the gene tree alignment tool. An additional 129 genes were identified using the MAKER-P metrics  $AED < 0.5 / QI2 [0.33 - 0.75]$ . Of those, 76 genes were excluded due to complex splice patterns. In the end, 12 genes were deemed suspicious by both approaches (gene tree visualization and P-MAKER metrics). Eighty-five genes curated and annotated in Apollo by the students are now being validated using RT-PCR. Annotation errors included missing exons, setting exon boundaries, identification of non-canonical splice sites or missing UTRs. Further tests are being performed to determine if these methods might be used to better predict gene models and develop workflows to automate the curation process.

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## Success in graduate education - Post-Baccalaureate program

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The gap in graduate educational attainment separating underrepresented minorities from other groups is particularly alarming in the Science, Technology, Engineering and Mathematics (STEM) fields. According to NSF, underrepresented groups account for only 13% of the Ph.D. population in STEM despite comprising 31% of the total U.S. population. Diverse cultural perspectives are known to inspire creativity and are drivers of innovation. Thus, developing approaches to increase the diversity and inclusion of all underrepresented groups in sciences is fundamental. Therefore, to increase the competitiveness and preparedness of historically underrepresented minorities in STEM fields for the rigors of graduate education, the Success in Graduate Education (SiGuE, <https://bmb.natsci.msu.edu/graduate-program/post-baccalaureate/programs/sigue/>) post-baccalaureate program was developed as a means to address this disparity in representation. The potential to advance knowledge upon which this research project is based has the long-term goal of gaining a comprehensive understanding of gene regulatory networks involved in the biosynthesis of phenolic compounds in maize. SiGuE consequently prepares its participants for graduate education via individualized development plans with an emphasis on gaining primary laboratory research experience in the integrated bioscience areas of genetics, molecular biology, biochemistry, and computational biology. Thus, didactic teachings encountered as an undergraduate are reinforced with research contributing towards a problem with real applications. Training for data presentation, written, and oral communication are also emphasized. As a result of this thorough post-baccalaureate training program, we have seen that the vast majority of SiGuE alumni have progressed to pursue and attain graduate education equipped with a solid background and experience in an active research environment. Therefore, initiatives such as SiGuE that provide a full immersion into practical research training in a nurturing personalized approach can provide an innovative opportunity towards increasing graduate level education of underrepresented groups.

Funding acknowledgement: National Science Foundation (NSF)

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## A deep learning approach to prioritize functional variants

(submitted by Hai Wang <[hw449@cornell.edu](mailto:hw449@cornell.edu)>)

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Prioritization of causal variants underlying agronomic traits is crucial for more efficient crop improvement. However, this is inherently difficult as it requires a mechanistic understanding of the information flow from genomic sequences to endophenotypes and, in turn, agronomic traits. To tackle this problem, we developed convolutional neural network (CNN) models for *ab initio* prediction of gene expression levels from genomic sequences, including a pseudogene model predicting binary gene expression levels (the area under the Receiver Operating Characteristic curve, auROC=0.94), an AGE model for Absolute Gene Expression levels (with R-squared between 0.4 and 0.5), and an ortholog contrast model predicting relative expression levels between species (auROC=0.81). Interpretation of CNN models, by saliency map, occlusion, and DeepLift, revealed an enrichment of DNA features important for gene expression in 5' and 3' UTRs. Cross-species predictions in maize, sorghum, and foxtail millet suggest that these DNA features are likely evolutionarily conserved. We further used the AGE model to predict the transcriptional effects of SNPs in the 282 panel, and found an enrichment of predicted large-effect SNPs around the transcription start sites. We also observed a significant enrichment of expression quantitative trait loci (eQTL) in predicted large-effect SNPs. In light of these results, we propose that prioritization of functional variants by deep learning models complements the limitations of traditional quantitative genetic approaches. This framework, combined with high-throughput genotyping, phenotyping, and genome editing platforms, will prove helpful for the upcoming *Breeding 4.0* era, where beneficial variants are rationally combined with unprecedented efficiency.

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

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## **A general framework to explain, model, and forecast trait of complex plasticity from diverse environments**

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The bottleneck of understanding phenotypic plasticity, the varied performance of the same genotype at different environments, is the quantification of relevant environmental stimuli from natural environments and their effects on plant growth. With extensive analysis of the multi-environment trial of a sorghum population with 237 recombinant inbred lines (RILs), we recently developed a general analytical framework, JGRA (Joint Genomic Regression Analysis), towards to overcome this bottleneck.

The RIL population were grown across 4 years at three fields spanning latitude from 18° to 42°. We recorded a complex flowering time pattern from seven tested environments. We discovered that photothermal time (PTT), an index integrating temperature and photoperiod, from 18-43 days after planting can be used to differentiate external stimuli of the environments due to its high correlation with mean flowering time. By leveraging PTT, the complex flowering time can be explained and predicted. The power of in-season and on-target prediction from this model was empirically validated with data from two new environments.

The JGRA framework was further tested with flowering time plasticity observed from a rice bi-parental population across nine environments. We are expanding the applications of the JGRA framework into in-season and on-target forecasting other traits from diverse accessions and elite cultivars in multiple crops.

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

P269

## **A genome-wide association study uncovers shared resistance mechanism between maize and sorghum to *Setosphaeria turcica***

(submitted by Zhang Xiaoyue <[xzhng128@illinois.edu](mailto:xzhng128@illinois.edu)>)

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Sorghum (*Sorghum bicolor* L. Moench), is a versatile C4 crop with high photosynthetic efficiency that is well-adapted to use as a biofuel feedstock. *Setosphaeria turcica* is an important plant pathogen, causing sorghum leaf blight on sorghum and northern leaf blight on maize. This pathosystem is useful for studying host specificity and investigating shared resistance mechanisms to *S. turcica*, as the same fungal species causes disease on both crops but isolates are host specific. We conducted a genome-wide association study (GWAS) in sorghum across multiple environments and consistently detected several regions associated with resistance to *S. turcica*. There were 75, 1050, and 537 significant ( $FDR < 0.01$ ) markers in the 2016, 2017, and the 2016 and 2017 datasets, respectively. A total of 111 unique genes were detected as candidate resistance genes. The most significant gene consistently detected across datasets was *CSLA4*, which is hypothesized to be involved in mannan synthesis. Additionally, we curated maize genes known to play a role in resistance to *S. turcica* and compared those with the association mapping results and found evidence of shared resistance genes between maize and sorghum. The maize gene and the corresponding sorghum homolog, *FAF-like* encoding-protein, were detected in both hosts. In Arabidopsis this gene has been shown to be involved in the negative regulation of abscisic acid-activated (ABA) signaling. We report novel candidate genes for resistance in sorghum and present evidence to support the hypothesis of shared resistance mechanisms between maize and sorghum.

Funding acknowledgement: Department of Energy (DOE)



P270 

## A GWAS investigation to reveal genes influencing the haploid induction rate of maternal inducers

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The production of doubled haploid lines (DHL) in maize relies on the use of haploid inducers. Haploid inducers are genotypes that have the inherent ability of generating seeds with haploid embryos when cross-pollinated to other maize plants. Haploid induction in maternal inducers is conditioned by a mutant allele of a pollen-specific phospholipase gene, named *MATRILINEAL* (Kelliher, 2017). However, the frequency of haploid seeds over the total number of seeds produced upon cross-pollination with a haploid inducer, commonly referred to as haploid induction rate (HIR), is known to be affected by multiple genes (Lashermes and Beckert 1988, Deimling 1997, Röber 1999). For instance, a QTL located on chromosome 9, named *qhir8*, was found to explain more than 20% of the genetic variance in a recent association study (Prigge et al. 2012), and had its effect confirmed and location narrowed-down in a subsequent fine-mapping investigation (Liu et al. 2015). HIR were raised from a frequency of 0.1% observed in natural maize populations (Chase, 1947) to 15% in most recent haploid inducers (Rotarencu et al. 2010). However, the genes responsible for this huge change in HIR have not been identified. Discovering their location and effect will make inducer breeding more efficient and consequently decrease the cost of DHL production. To reveal these genes, we conducted a genome-wide association study (GWAS) using the HIR information generated by pollinating a single donor genotype with 187 different haploid inducers, in the summer of 2018. Genotypic data was produced using Diversity Array Systems Technology (DArTSeq) markers. In this poster, we describe the methodology used in this investigation, expose the polymorphisms identified and discuss how they can be used to increase the efficiency of inducer breeding.

Funding acknowledgement: National Council for Scientific and Technological Development (CNPQ - Brazil)

P271 

## A novel platform provides improved estimation of stalk lodging resistance in maize

(submitted by Rajandeep Sekhon <[sekhon@clemsun.edu](mailto:sekhon@clemsun.edu)>)

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Stalk lodging causes significant yield losses in maize and other grasses worldwide. Stalk lodging resistance (SLR) is a complex trait attributable to several anatomical, biochemical, and physiological intermediate phenotypes. Deciphering the genetic architecture of SLR and the development of breeding metrics to improve this trait have been seriously hindered by the lack of a robust phenotyping platform capable of replicating the “real-life” stalk lodging phenotype in test plots. Traditionally used SLR phenotyping methods like lodging incidence count and rind penetration resistance (RPR) are prone to inconsistent testing parameters, heavy environmental influence, and one-dimensional analysis. We have deployed a novel phenotyping platform, developed with extensive insight from structural engineering that evaluates the ability of individual maize stalks to resist bending loads before failure (i.e. bending strength). We have also identified putative key intermediate traits underlying SLR to gain insights into their relative impact on SLR. To evaluate the new platform and the contribution of intermediate traits to SLR, we recorded bending strength, RPR, and other intermediate traits of interest on a subset of maize hybrids in the Genomes to Fields (G2F) initiative. Using the historical lodging data generated by the G2F initiative, we demonstrate that bending strength is a strong predictor of SLR. This data also revealed interesting relationships among the intermediate traits and provided novel insights into the structural and anatomical features that contribute to SLR. The improved platform and phenotyping approach provide a reliable estimation of SLR for accelerating breeding efforts and deciphering the genetic architecture of SLR in maize and other grasses.

Funding acknowledgement: National Science Foundation (NSF)

P272 

## **A practical haplotype graph enables low cost imputation and haplotyping of diverse maize populations**

(submitted by Jose Valdes Franco <[jav246@cornell.edu](mailto:jav246@cornell.edu)>)

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To cost-effectively genotype large maize populations, the genome has to be analyzed through some form of reduced representation of its sequence (e.g., GBS, rhAmpSeq). When combined with maize's high level of diversity and its highly repetitive sequence, this type of data represents a challenge for genotyping and imputation. This is particularly the case when generating the haplotype-level data necessary to best identify causal genomic regions controlling for maize's ability to grow in a broad range of environments. Maize germplasm banks, representing the vast environmental adaptational diversity, present a golden opportunity to identify the genomic regions regulating this adaptation.

Here we present a Practical Haplotype Graph (PHG) approach which enables the low-cost imputation and haplotyping of diverse maize populations by creating a database of haplotypes from existing maize assemblies over a set of reference intervals. An implementation of the PHG is being tested to impute genotypes for the accessions in the NAM population and the Genomes to Fields (G2F) and Seeds of Discovery (SeeD) projects. The PHG's ability to impute inbred material, which shares a close relationship to the assembled genomes included in the database, shows a similar accuracy the standard imputation approach. We expect to improve upon the standard approach by including assemblies more closely related to the more diverse landrace material.

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

P273 

## **Allelic diversity and combining ability among expired plant variety protection maize stiff stalk inbreds**

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Commercial maize breeding has exploited heterosis to produce hybrids that support U.S. and international crop production. Breeding programs within independent companies produced selection signatures that underlie the outcomes of the directed breeding efforts. The availability of commercial inbred lines with an expired Plant Variety Protection (ex-PVP) certificates provide an opportunity to assess the allelic changes that have occurred through the hybrid breeding process. A panel of 328 expired-PVP inbred lines, representing the parents of widely-grown hybrids in the 25 years prior to the transgenic era, was genotyped at 899,784 SNPs derived from RNA-sequencing. Hierarchical clustering and ADMIXTURE analyses grouped these expired-PVP lines based on genetic similarity into eight heterotic sub-groups. Minor allele frequency,  $F_{st}$ , and Tajima's D were used to assess genetic diversity on a chromosomal basis for each heterotic sub-group. Tajima's D indicated differential reduction of genetic variation as a result of selection or recent population expansion for the individuals in the Iodent and B73 sub-groups on chromosomes two, five, and seven. These haplotypes are hypothesized to play an important role in complementary gene action of overdominance, additive-additive epistasis, and pseudo-overdominance which drives hybrid vigor. Using pedigree information provided in PVP certificates, heavily recycled Stiff Stalk progenitors were identified which contributed in the generation of elite stiff stalk female inbreds used in the generation of commercial hybrids today. To explore the effect of crossing between and among heterotic sub-groups, 540 unique hybrids derived from expired-PVP inbreds were evaluated in a multi-environment yield trial experiment. Highly heterotic crosses were identified among the B73/Iodent, Oh43/Iodent, and B37/Iodent-Lancaster sub-groups. These results define the heterotic composition and diversity present for the current set of publicly available expired-PVP germplasm, which can be used to greatly expedite the process of utilizing this set of elite germplasm for the improvement of maize

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Dave and Sharron Mies Fellowship

P274 

## **An integrated phenomics approach to identifying the genetic basis for maize root structure and control of plant nutrient relations**

(submitted by Christopher Topp <[ctopp@danforthcenter.org](mailto:ctopp@danforthcenter.org)>)

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The goal of the project is to identify the genetic basis of maize root architecture and its relationship to soil nitrogen availability, plant nitrogen status, and the elemental content of the seed. The project will compare four different root phenotyping methods: 1. 3D gel imaging, 2. X-ray CT, 3. Root crown excavations, and 4. Minirhizotron analysis, with the seed ionome and other above-ground traits. Experiments will focus on two major sets of genetic material: **1. The Illinois Protein Strain Recombinant Inbred (IPSRI) population, consisting of ~500 lines that were generated by a cross between the Illinois High Protein (IHP) and Illinois Low Protein (ILP) parental populations, and 2. Two diverse hybrid populations - Goodman-Buckler Diversity Panel and Teosinte Synthetics – crossed to PHZ51.** The information collected from these experiments will be used to compare the four root phenotyping methods, the relationship of root structure, nitrogen plasticity, and the seed ionome, and to identify QTL and the genes controlling them. Ultimately, this research could lead to improvements in our ability to grow plants with fewer nutrient inputs and reduce the environmental costs of agriculture. Our proposed educational activities are collectively designed to help plug “leaks” at a number of key educational stages. The program will include (1) an ASPB-funded outreach program at an Urbana middle school that will also be expanded to a St. Louis middle school; (2) research opportunities for high school and undergraduate students and (3) a DIY phenomics and 3D printing workshop for high school ‘Maker’ groups in St. Louis.

Funding acknowledgement: National Science Foundation (NSF)

P275

## **Ancient samples in quantitative trait analysis**

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Researchers have long recognized that flowering time in maize is highly correlated with other agronomically important traits through shared population structure. We test the use of ancient samples in quantitative analysis of flowering time, a trait for which selection drives population differentiation.

Funding acknowledgement: National Science Foundation (NSF)

P276

## Assessment and visualization of haplotype structure diversity among commercial maize ex-PVP lines in relation to key founders

(submitted by Stephanie Coffman <[stephanie.coffman@pioneer.com](mailto:stephanie.coffman@pioneer.com)>)

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Development of elite proprietary inbreds among private seed industry companies has been a major driver of increased genetic gain in North American maize hybrids over the last several decades. The history of these industry inbreds can be traced back to important founder lines, some of which were key contributors to the development of heterotic groups. Pedigree-based analyses in previous studies have been used to summarize genetic diversity and population structure among ex-PVPs, however, these rely on records that are sometimes unavailable or inaccurate. Marker data have been used to assess genetic diversity among ex-PVPs often as part of larger diversity studies, but less is known regarding the extent of haplotype sharing between particular ex-PVPs and their founder lines. Taking advantage of LD captured by multi-SNP haplotypes can provide a more accurate assessment of the relationships between inbreds with shared ancestry compared to single marker comparisons. We performed high-density haplotype analysis with 11.3 million SNPs on 212 maize inbreds including 157 ex-PVPs and 55 public lines. We focused on haplotype sharing with 12 key founders identified through literature review: 207, A632, B14, B37, B73, LH123HT, LH82, Mo17, Oh43, OH7, PHG39 and Wf9. Our results reveal significant haplotype sharing between these ex-PVPs and founder lines, uncover source variation and quantify similarities and contrasts between heterotic groups and the major U.S. seed industry companies. These results increase the resolution of our understanding of industry germplasm breeding history, demonstrate the effectiveness of haplotype sharing as a tool to assess germplasm diversity and provide information which can help inform line selection in studies of heterosis, trait-associate haplotypes and breeding program diversity. An R/Shiny application has been developed to provide an interactive interface facilitating exploration of these whole genome haplotypes by inbred and comparisons across inbreds. This tool will be available through the MaizeGDB website.

P277 

## Breeding for natural food and beverage colorants from corn

(submitted by John Juvik <[juvik@illinois.edu](mailto:juvik@illinois.edu)>)

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Color has a significant impact on the perception of food and beverage quality, playing an important role in its marketing, desirability, and consumption. Consumers are interested in replacing artificial food dyes with natural alternatives mirroring a larger shift in consumer preference for less processed foods. Current sources of natural pigment such as purple carrot, beet, and radish are expensive and inefficient, producing byproducts with little to no value. Current pigment sources will likely be unable to meet growing market demand. Purple corn contains the same pigments currently used for natural dyes. Purple corn can be grown in place of yellow corn, allowing for a simple transition for growers and a product that is scalable and therefore capable of meeting market demands. Established processing methods allow pigment extraction while reserving the remainder of the kernel for use in food, feed, or fuel, creating a value-added product that can be grown for a premium.

Funding acknowledgement: United States Department of Agriculture (USDA)

P278 

## Breeding maize with nitrogen in mind for ethanol production

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Ethanol production relies on inexpensive carbohydrates. In the United States, maize is the primary carbohydrate source; in 2017, over 40% of US maize went to ethanol production. 60-90% of the maize production greenhouse gas (GHG) contribution stems from nitrogen fertilizer application, with 48–64% as N<sub>2</sub>O emissions from soil and stover decomposition and the rest from nitrogen synthesis. To reduce ethanol production's GHG footprint N<sub>2</sub>O emissions must be reduced. We propose a two fold solution: Nutrient remobilization and reduction in kernel protein.

Maize has perennial and semi-perennial wild relatives in *Zea* and *Tripsacum* that likely produce strong sinks for end-season nitrogen. We will study this natural diversity to understand strategies for remobilizing >75% of maize nitrogen to below the soil in forms largely unavailable for nitrification/denitrification into N<sub>2</sub>O. This could reduce N<sub>2</sub>O emissions and fertilizer use. Hybrids with low-protein/high-starch kernels, when coupled with remobilization, have the potential to vastly reduce nitrogen inputs, as most nitrogen would remain in the field. This could increase profits for producers, both by increasing ethanol yields and reducing byproduct drying costs. Kernel protein is heritable and selectable, though it may reach a lower physiological limit at ~5% protein, as protein is essential for seedling vigor. However, within maize production, only the seed planted has to have enough protein to germinate, not the seed harvested. As such, we will breed for and study low-protein hybrids. We will present: A breeding plan for the creation of low-protein maize, along with the assumptions, calculations, and results of an economic model using a nutrient remobilizing, low-protein maize.

Overall, if both nitrogen remobilization and low-protein can be achieved, combined, and deployed into a hybrid system it could greatly reduce the GHG emissions of maize ethanol production, as well as reducing other environmental problems arising from nitrogen application.

Funding acknowledgement: United States Department of Agriculture (USDA)

P279 

## Characterizing and fine-mapping a mutation in maize which confers reduced leaf area

(submitted by Marlee Labroo <[labroo2@illinois.edu](mailto:labroo2@illinois.edu)>)

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A key driver of historical increases in US maize yields is improved varietal tolerance of high planting densities, which is strongly correlated with reduced leaf area. A maize mutation conferring reduced leaf area, *rdla*, may be useful in targeted improvement of yield at high planting densities and narrow row spacing. Initial characterization of the mutation at the BC5S10 generation in an Oh43 background suggests that the reduced leaf area phenotype is only apparent in adult leaves, and adult leaf area of *rdla* genotypes is reduced by approximately one-third compared to wild-type. The *rdla* mutants show more moderate reduction in leaf area than known leaf area mutants in the Maize Genetics Coop Stock Center, and complementation tests show that the *rdla* mutation is distinct from the known mutants. Preliminary data suggest that average cell size of adult leaves differs in *rdla* genotypes compared to wild-type, which may be the anatomical underpinning of the reduced leaf area phenotype. We mapped the *rdla* mutation to an ~80 Mb region on chromosome 4. Using imputation with an estimated accuracy of 94%, the location was further narrowed to a 4 Mb subregion. Based on our mapping results, we generated markers to facilitate backcrossing of *rdla*, a recessive mutation, into diverse genotypes. The diverse genotypes can later be used to assess *rdla* phenotype penetrance in both inbred and hybrid backgrounds. In the future, we also plan to quantify the difference in cell size between *rdla* and wild-type genotypes in replicated trials and to fine-map the underlying *rdla* mutation via differential expression analysis and generation of a mapping population.

Funding acknowledgement: United States Department of Agriculture (USDA)



P280

## Comparison of sampling strategies to maximize accuracy of genomic prediction in maize landraces

(submitted by Armin C. Hoelker <[armin.hoelker@tum.de](mailto:armin.hoelker@tum.de)>)

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Harnessing novel variation from maize landraces requires intensive pre-breeding efforts to close the performance gap between landrace-derived material and elite germplasm. Genomic prediction (GP) can assist in accelerating pre-breeding, if high-precision phenotypic and genotypic data are available for training the prediction model. Obtaining high-quality phenotypic data for maize landraces is challenging due to their heterozygous and heterogeneous nature. Here, we compare two different strategies for sampling individual gametes from landraces and generating reproducible genetic units for phenotyping and training of the prediction model.

From each of two European landraces we generated a library of doubled-haploid (DH) lines and a library of gamete capture (GC) lines. Fully homozygous DH-lines were produced by *in-vivo* induction, such that the lines of the DH-libraries represent individual gametes of the landrace. For production of GC-lines, pollen of the landrace was used to fertilize an elite inbred line. These crosses produced progeny consisting of a landrace gamete and the elite inbred line gamete. Two subsequent selfing steps were applied to obtain partially homozygous lines. The resulting 1421 lines (873 DH, 548 GC) were genotyped (600k SNPs) and phenotyped for line *per se* performance in adjacent field trials in two locations and two years for cold-related, morphological and agronomic traits.

We compared prediction accuracies for the two different sampling strategies within and between libraries from the same landrace. Genomic prediction accuracies strongly depended on the landrace and the trait under study. In most cases, prediction accuracy was highest within the DH-libraries. Across-library prediction (DH on GC or vice versa) resulted in low to moderate (0.1-0.4) prediction accuracies. We will discuss our findings with respect to efficient genomic pre-breeding strategies of landrace-derived genetic material for improvement of elite germplasm.

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## CREAMD (Core Root Excavation using Compressed-air) COFE (Core Root Feature Extraction) for high throughput root phenotyping for field grown plants

(submitted by Stefan Hey <[shey@iastate.edu](mailto:shey@iastate.edu)>)

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Unveiling the genetic control of RSA (root system architecture) in crops via large-scale GWAS (genome-wide association study) requires high-throughput pipelines for root collection and phenotyping. Classic root washing and scoring methods are time consuming and resource intensive. To increase throughput, we developed a high throughput pipeline for root phenotyping of field grown plants. CREAMD (Core Root Excavation using Compressed-air), uses the AirSpade®, a pneumatic tool to apply compressed air for root excavation and cleaning. Subsequently, COFE (Core Root Feature Extraction), a semi-automated software, was used to extract traits from high resolution 2D root images. We extracted six traits based on systematic heritability testing. Roots were imaged from two different views (North and West based on their orientation in the field). More than 5000 roots of maize and a sorghum were excavated from diversity panels. Phenotypic values obtained via CREAMD are comparable to those obtained via classic root washing methods, while significantly reducing time and resource requirements.

Funding acknowledgement: Department of Energy (DOE)

P282 

## Crop growth model calibration and simulation of 12 maize hybrids

(submitted by Cassandra Winn <[cwinn@iastate.edu](mailto:cwinn@iastate.edu)>)

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Crop modeling approaches that combine environmental parameters, management, and maize hybrid properties have been described to predict genotype by environment interactions (Technow et al., 2015). Plant breeders have the potential to be greatly supported by crop models. However, large-scale application of crop modeling in plant breeding is severely limited by labor and cost required to measure cultivar-specific crop-model parameters. The use of crop models in plant breeding has primarily been for identifying and improving physiological traits. Typically, these studies don't measure near the number or range of genetic coefficients needed to fully parameterize a crop model. In fact, many studies classify varieties into maturity groups, and hybrid-specific coefficients are assumed to be analogous within each maturity group (Yang et al., 2004; Archontoulis et al., 2014). Our objective was to determine what parameters differentiate 12 commonly grown central corn belt hybrids and if differences in yield, phenology, biomass accumulation and partitioning, and nitrogen uptake can be accurately simulated from a limited set of parameters. In this experiment, the APSIM (Agricultural Production Systems sIMulator) maize model was calibrated for 12 hybrids grown widely throughout the Genomes To Fields Initiative by empirically estimating hybrid-specific model coefficients from field experiments. After calibration, simulations of crop growth were conducted and a difference between hybrids was clearly expressed in traits such as biomass accumulation, partitioning, and nitrogen uptake.

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

P283 

## Cross-species validation of maize GWAS candidates for root architecture

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Candidate genes controlling root architecture were identified through a genome wide association study (GWAS) in the maize recombinant inbred lines, IPSRIs (Illinois Protein Strain Recombinant Inbreds). However, GWAS relies exclusively on statistical association, so functional validation is necessary to make strong claims about gene functions. Functional validations of our highest-priority gene IRA1 (Ideal Root Architecture 1) were performed across multiple species: maize, tomato, and Arabidopsis. We compared sequence and gene expression variation between inbreds representing the parental populations, and among RILs with extreme phenotypes at the QTL. Increases in lateral root numbers are correlated with higher gene expression levels of IRA1. RNAi lines of an IRA1 tomato ortholog were phenotyped on our 3D root imaging platform. Several traits involving lateral roots and the volume of soil exploration varied greatly between wild type and RNAi lines. Similarly, T-DNA insertions in an Arabidopsis ortholog of IRA1 led to dramatic reductions in RNA levels that were correlated with both shorter primary roots and fewer lateral roots. All lines of evidence suggest IRA1 has a major role in root system development and quantitative genetic control of root architecture in maize. Genetic manipulation by CRISPR/CAS9 in maize is in progress.

Funding acknowledgement: National Science Foundation (NSF)

P284

## **Demonstration of local adaptation of maize landraces by reciprocal transplantation**

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Populations are said to be locally adapted when they exhibit higher fitness than foreign populations in their native habitat. Examples of maize landrace adaptations to highland and lowland conditions are widely reported, and are of interest to researchers and breeders. Although landraces are commonly believed to be locally adapted, there is little empirical evidence to support this intuition. To determine the prevalence and strength of maize landrace local adaptation, we performed a reciprocal transplant experiment across an elevational gradient in Mexico. We grew 120 landrace accessions from North and South America in highland and lowland Mexican common gardens and collected phenotypes relevant to fitness, including the reported highland-adaptive traits anthocyanin stem pigmentation and macrohair density.

We find phenotypic patterns consistent with local adaptation, though these patterns differ between Mexican and South American populations. Generally, Mexican landraces had higher fitness than South American landraces, and landraces generally exhibited higher fitness when grown near their native elevation. Highland populations expressed higher anthocyanin pigmentation than lowland populations, and this difference was more pronounced in highland sites than in lowland sites. Macrohair density was largely non-plastic with Mexican and highland landraces generally being more pilose. Analysis of d13C and d15N revealed that lowland populations have lower water use efficiency and are less discriminatory against 15N in nitrogen uptake. Each population has garden-specific correlations between highland trait expression and fitness metrics, with highland populations having stronger positive correlations in the highland site.

These results give substance to the long-lived presumption of local adaptation in New World maize landraces, both to elevation and to additional environmental variables distinguishing North and South America.

Funding acknowledgement: National Science Foundation (NSF)

P285

## **Development of a genetic screening system to characterize maize nodal root growth responses to water deficit**

(submitted by Nicholas Baert <[nwbbgk@mail.missouri.edu](mailto:nwbbgk@mail.missouri.edu)>)

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Drought is the leading abiotic stress factor limiting crop productivity worldwide, and climate projections predict droughts that are more widespread and increased in their intensity. It has been demonstrated that maize nodal roots have a superior ability to continue elongation under water-limiting conditions relative to other plant organs. Genetic variation in the extent of this response exists, though the mechanisms involved in this growth maintenance are largely unknown. Maize genotypes with a greater ability to maintain nodal root growth through dry soil will be better able to access water at depth, and thus may have a competitive advantage over genotypes with a weaker nodal root growth response. To study the underlying mechanisms reproducibly across a range of genotypes, a plant growth system was designed that allows for the characterization of nodal root phenotypes in 5-week old water-stressed maize plants. Utilizing a dry-down (withholding water) approach with plants grown in 122-cm deep tubes, we can consistently achieve media water potentials that elicit characteristic water-stress responses in maize plants. This system facilitates greater screening throughput, and allows us to study more whorls of nodal roots than existing methods used to characterize nodal root growth responses to water deficits. Going forward, we will characterize genetic variation (e.g., select mutants, inbred-hybrid trios, NAM parents, etc.) in nodal root growth responses to water-stress in order to elucidate the genetic mechanisms involved in root growth maintenance.

Funding acknowledgement: National Science Foundation (NSF)

P286

## Disentangling the role of copy number variants in the phenotypes of doubled-haploids derived from European maize landraces

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Copy number variants (CNVs) are structural rearrangements, resulting from the gain or loss of specific DNA regions. CNVs lead to variations among the genome of individuals not captured by single nucleotide polymorphisms (SNPs). They have been associated with phenotypic variation in animals and plants, including maize. In the present study, we detected more than 6000 CNVs in ~ 1000 doubled-haploid (DH) lines derived from three European maize landraces using SNP chip intensity data. The landrace material allows detecting diversity not present in European elite material. CNVs will be validated by molecular techniques, genome and scaffold assemblies, as well as with read depths of whole genome sequences derived from a subset of the DH-lines. We show the association of the detected CNVs with important maize agronomic traits, evaluated in two consecutive years in different environments in Europe, as well as root traits assessed in controlled environments in cold and normal conditions.

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## Dissecting the genetic architecture that controls the domestication of maize leaf morphology

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From its wild progenitor teosinte (*Zea mays* ssp. *parviglumis*), maize has experienced a dramatic morphological transformation. Although significant advances have been achieved in the identification of the genes that control the changes in overall plant architecture during domestication, the genetic basis that controls the changes in leaf morphology, an important component of plant architecture, remains poorly understood. Here, using a large population of 866 maize-teosinte BC2S3 recombinant inbred lines genotyped with 19838 SNP markers, we performed high-resolution quantitative trait locus (QTL) mapping for three leaf morphological traits, including leaf length, leaf width, and sheath length. We demonstrate that the three leaf traits were associated with distinct genetic architecture features and under relatively independent genetic control. This genetic independence was further validated by the analysis of near isogenic lines for target QTLs. QTL characterization revealed that the three leaf traits might have experienced directional selection for increased leaf size during maize domestication. We found that known leaf development genes identified by mutagenesis were significantly enriched in the support intervals of leaf trait QTLs, potentially indicating their important roles in regulating the natural variation in leaf traits. Our findings provide novel insights into the genetic basis that controls maize leaf evolution.

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**P288**

### **Dissecting the genetic control of plant height in *Setaria***

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Plant architecture is the composite product of genetic and environmental factors as well as their interaction across the development or life cycle of the plant. We have been studying plant architecture in a recombinant inbred line population developed from a cross between a *Setaria italica* and a *S. viridis* accession. A genomic region on chromosome 5 consistently displayed a strong effect on plant height and branching. QTL for these two essential components of plant architecture were identified in this region across ten different trials, particularly for tillers at flowering time. The QTL region was located between 38,270,947 and 43,240,044 bp on chromosome 5 (~4.969 Mbp), and comprised about 719 genes. All physical positions are based on the published *S. viridis* genome. A recombinant inbred line identified as heterozygous in the region between 38,804,111 and 42,099,133 was used to develop heterogeneous inbred families (HIFs). Several HIFs were evaluated both genetically and phenotypically, and the region of interest, particularly for height, was shortened to ~380,000bp (between 41,719,133 and 42,099,133 bp). This reduced region of interest encompasses ~54 genes, some of them having alternative splicing transcripts. We present here some preliminary RNA analyses, including RNA-seq from leaves and internodes of HIFs with alternate alleles for this region, and qPCR of several promising candidate genes.

Funding acknowledgement: National Science Foundation (NSF)

**P289**

### **Divergent selection for shoot apical meristem (SAM) size induces correlated changes in adult plant phenotypes**

(submitted by Aaron Kusmec <[amkusmec@iastate.edu](mailto:amkusmec@iastate.edu)>)

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The shoot apical meristem (SAM) is responsible for the development of all aboveground organs in plants. Previous research demonstrated that SAM volume in maize correlates with agronomically important adult plant traits including height to primary ear and days to anthesis. To further investigate these correlations, we bred two populations of maize divergently selected for large and small SAM volumes. 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. After three cycles of selection for increased or decreased SAM size, doubled haploid lines (DHLs) were produced. These DHLs were phenotyped for SAM volume to determine the efficacy of genomic selection and for adult plant phenotypes to examine correlated selection responses. These populations will serve as a resource for further analyses of the relationship between SAM volume and adult traits, as well as potential limits to selection.

Funding acknowledgement: National Science Foundation (NSF)

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## Environmental proxies and genomics allow for in-season prediction in exotic-derived maize lines

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Since its spread during domestication from teosinte (*Zea mays* ssp. *parviglumis*) in the Balsas river valley of Mexico, maize (*Zea mays* ssp. *mays*) has become adapted to a large latitudinal range of environments, with flowering time being one of the most important adaptive traits. A human-mediated dispersal of maize, following domestication, led to the large amounts of diversity seen in modern maize. Currently, maize is used extensively for the production of food, feed, and fuel; however, trends show maize's current and projected yields to fall short of the world's future demands. Climate change, with growing drought and heat stress episodes across regions like the U.S. Corn-Belt, can further inhibit the already insufficient yield growth trends. Exotic maize germplasm serves as a potential source of genetic variation to overcome yield gaps and climatic changes, but the pre-breeding processes needed to adapt this germplasm to the U.S. Corn-Belt is limited in scope. Herein, 178 doubled haploid lines, derived from 53 exotic maize germplasm accessions and adapted through backcrossing the exotic germplasm accession to elite lines, were studied for stability of flowering-related traits. Ten environments, spanning 30°N to 42°N latitude, were used to extensively phenotype the lines. The incorporation of an environmental index, constructed from temperature and day-length data, was used to quantify these environments. This environmental index was found to be highly correlated with the environmental mean ( $r = -0.95$ ). Through the infusion of this environmental index and genomic data from the doubled haploid lines, in-season and on-target predictions for new environments were obtained. These accurate predictions will allow for the identification of those lines with stable performance and increase the overall efficacy of adaptation to specific, targeted environments.

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

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## Establishment and evaluation of non-destructive selection method on single kernel moisture based on NMR in maize

(submitted by Ming Chen <[acm2638@163.com](mailto:acm2638@163.com)>)

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Maize kernel moisture is one of the most important issues affecting mechanical harvest in maize. A high moisture of kernel during harvest stage leads to high breakage ratio and high mildew rate. Difficulties exist in the kernel moisture selection, which including the inaccuracy and lack of the fast and non-destructive methods. The biggest problem needed to be solved in the kernel moisture selection is the inaccuracy of the measure method which demands to be fast and non-destructive. Here we established a fast and non-destructive method which accurate the determination of maize kernel moisture based on nuclear magnetic resonance (NMR). Compared to oven drying method, our method could keep the measurement bias under  $\pm 1\%$  in the moisture region from 18% to 37%. Single kernel moisture selection towards high and low directions in hybrids, the result of bidirectional selection on single kernel moisture with our method showed that, the average moisture difference between high moisture population and low moisture population in F5 was 15.09%, which was significantly higher than that of 4.93% in F2 generation, indicating the effectiveness and high efficiency of NMR based method. Meanwhile, some agronomic traits of the populations with high moisture and low moisture also showed separation, the population with low kernel moisture population showed lower 100 kernel weight, thinner bracts and leaves. In conclusion, we established a highly efficient and non-destructive single kernel moisture selection method in maize, which could play a great role in kernel moisture research and breeding programs in maize.

Funding acknowledgement: The National Key Research and Development Program of China (2016YFD0101201), the Modern Maize Industry Technology System (CARS-02-04)



P292

## Evaluation of a panel of maize lines for resistance to fall armyworm

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The fall armyworm (FAW, *Spodoptera frugiperda*), is a non-diapausing, generalist lepidopteran herbivore endemic to tropical and sub-tropical North America. The larvae feed on over 300 plant species, including nearly all major crop plants, and especially prefer the Poaceae. In maize, FAW caterpillars cause severe yield losses primarily by early-season defoliation that reduces photosynthesis. However, late-emerging larvae often burrow into the developing ear, causing direct damage and allowing pathogens access to the grain. Naturally FAW-resistant pedigrees have been developed by phenotypic mass selection under FAW pressure, but many of these were bred using a narrow genetic base of subtropical germplasm. FAW can be reasonably expected to expand its range into more temperate areas if climate change occurs as predicted, thus necessitating the development of novel FAW-resistant temperate pedigrees. Alarmingly, between 2016 and 2019 FAW further expanded its range into all of sub-Saharan Africa, Yemen, India, Sri Lanka, and Bangladesh, thereby posing an imminent threat to eastern Asia in 2019. Although genetically-modified (GM) *Bt* pedigrees can be effective in combating FAW, GM food crops are currently banned in many of these newly colonized countries. Thus, non-GM, FAW-resistant inbred breeding stocks should be identified that also provide superior combining ability in F1 progeny hybrids. Here, we present the results of a screen for FAW resistance using a panel of maize inbred lines we developed, conducted in parallel with fifty-eight of their F1 hybrid progeny lines. Families were evaluated at Mississippi State University for FAW resistance in June 2018, and subsequently scored for other agronomically valuable traits in a temperate environment in field trials at Cornell University. Taken together, these trials will identify breeding stocks that are naturally adapted to a wide variety of growing conditions, which farmers may ultimately use to mitigate FAW herbivory and enhance maize yields in a site-specific manner.

Funding acknowledgement: United States Department of Agriculture (USDA)

P293

## Evaluation of strategies for simultaneous prediction of three correlated maize architectural traits

(submitted by Brian Rice <[brice6@illinois.edu](mailto:brice6@illinois.edu)>)

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Multi-trait methods that use information from correlated traits measured within specific plant and animal species are an emerging focus of quantitative genetics. Genomic selection and genome-wide association studies (GWAS) are traditionally performed univariately on a single trait; however analyses that also include correlated or “proxy traits” are an important economic tool for prediction if they are less resource intensive to measure than the trait(s) of interest. Although promising, this area of study requires further evaluation across a wide variety of genetic architectures and species. Therefore, we studied three maize architectural traits (leaf angle, ear row number and tassel branch number) that have been shown to be associated with putatively pleiotropic genomic loci. To aid in distinguishing between pleiotropic and non-pleiotropic loci, we are currently developing an analytical pipeline that utilizes the simultaneous application of multivariate and univariate statistical models to associate genomic markers with these traits. The data set we will analyze will contain measurements from these three traits in nearly 2,000 diverse maize genotypes across multiple field seasons. In addition to facilitating the identification of genomic regions that potentially harbor pleiotropic loci, we hypothesize that this data set facilitate accurate modeling of genetic and non-genetic sources of trait variability. These data will also be used for evaluating an approach for multi-trait genomic prediction where genomic markers associated with peak GWAS signals from one trait are set as fixed effect covariates in a Ridge Regression Best Linear Unbiased (RR-BLUP) model for the prediction of a correlated trait. Description of the genetic architecture and the correlation of these maize traits will add to the growing area of multi-trait quantitative genetics.

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## Expanding european flint maize panels for genome wide association and genomic selection studies

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European flint maize has proven to be an outstanding partner of North American dent maize for developing hybrids in Northern Europe. Use of publicly available genetic material in genetic studies is nevertheless complicated by a number of agronomic defaults, especially root and stalk lodging, tillering and a number of disease susceptibilities. A first series of lines available at INRA (Camus-Kulandaivelu et al. 2006, Genetics), was increased within the CornFed project, which involved German, Spanish and French partners, leading to 300 diverse lines. These lead to the discovery of QTL for biomass production (Rincent et al., 2014, Theor Appl Genet) and cold tolerance (Revilla et al., 2016, BMC plant Biology). To further increase this panel, Gouesnard et al. (2017, Theor appl Genet) conducted a GBS based survey of all flint type materials present in the INRA collection. Original lines not introgressed by dent germplasm were selected to complete the collection. They were also intercrossed to produce new recombinant materials between different genetic origins. This led to a total of 1200 preexisting and new lines that were evaluated *per se* in a windy environment in Brittany to favor lodging, which revealed a high genetic variability. Association genetics will be conducted in the coming months. Finally, out of these lines, 350 lines selected within a narrow phenology range and showing limited lodging susceptibility have been crossed to a dent tester to conduct agronomic trials for adaptive traits, to be started in 2019.

Funding acknowledgement: INRA, ANR-10- BTBR-01 (Amaizing), France Agrimer

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## Exploring plant height plasticity observed in natural environments

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Plant phenotypes are determined by genetics, environment, and their interactions. Phenotypic plasticity describes that a genotype behaves differently when exposed to different environments. When multiple genotypes are showing different levels of phenotypic plasticity, genotype by environmental interactions (G x E) are present. Unraveling G x E is crucial to understanding plant local adaptability, which can be utilized in breeding, and provide new solutions in times of climate change. Recently, we established a joint genomic regression analysis (JGRA) framework to dissect the complex flowering time plasticity observed in natural environments by leveraging an explicit environmental index. In this study, we hypothesized that plant height G x E interactions can be unraveled and explained in a similar manner. The objectives were to 1) uncover the patterns of sorghum plant height plasticity in diverse environments; 2) predict performance in new environments; 3) identify and dissect the genetic determinants to explain the observed G x E interactions. Our results indicated that varied degree of plasticity in plant height of sorghum lines could be explained, modeled, and predicted with a biologically meaningful environmental index. High prediction accuracy ( $r = 0.88$ ) was achieved by using this environmental index. The effects of three height QTLs changed dynamically across environments, contributing to the observed G x E. In conclusion, by combining environmental and genomic components, we were able to explain and predict sorghum plant height under natural conditions.

Funding acknowledgement: United States Department of Agriculture (USDA), Iowa State University Plant Sciences Institute; Iowa State University Raymond F. Baker Center for Plant Breeding

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## Exploring the genetic regulation of gene expression in maize roots

(submitted by Guangchao Sun <[gsun2@unl.edu](mailto:gsun2@unl.edu)>)

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The roots of maize, or indeed any plant, face a biological challenge in that they must perceive and respond to their environments using decision logic which is hardwired at a genetic level. Here we seek to create a directed edge transcriptional network model for maize roots using expression GWAS. Lines were selected from either the 282 Buckler-Goodman association panel composed primarily of temperate adapted lines which can be cultivated in Nebraska or a set of an additional panel of ~250 tropical adapted maize inbreds from the National Plant Germplasm Service capturing a great deal of allelic diversity absent from temperate maize. To date, we have processed a total of 594 RNA-seq libraries, representing one or more replicates of root tissue from 352 distinct maize genotypes. Reads were aligned to the B73v4 reference genome for both expression profiling and SNP calling. A set of 26,343 genes which exhibit sufficient expression (expressed in 80% of the population) were used for downstream analysis and mapping. Individual genes were binned based on their broad sense heritability of their expression levels among the assayed samples with multiple replicates. Functional enrichment analysis indicated that genes with higher expression heritability tended to be involved in core metabolism and cell cycle regulation while genes with lower expression heritability were enriched in a wide range of functions involved in perception and response to the environment. 26,343 eGWAS runs with two models -- FarmCPU and MLM -- were accomplished and analyzed. 15,419 TASs (Trait Associated SNPs) showed significant impact on the expression level of 5,071 individual genes and a putative eQTL hotspot on Chromosome 10 in which 442 TASs are located in a small interval containing 80 annotated genes was identified. Future work will be focused on constructing regulatory models for transcription by integrating eGWAS results, co-expression, and metabolic networks aiming to uncover molecular mechanisms underlying maize root development and its interactions with abiotic-/biotic spheres.

Funding acknowledgement: National Science Foundation (NSF)

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## Exploring the hidden half: sequence variation in maize orthologs of *Deeper rooting 1 (Dro1)* and root architecture differences in the genus *Zea*

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Understanding the genetic control of root architecture holds untapped potential that could be harnessed for future breeding efforts. To date, only two genes controlling root architecture have been cloned: *Pstol1* and *Dro1* in *O. sativa*. *Dro1* was identified in *O. sativa* landraces with steeper root angles and has been shown to increase yield under drought conditions. Three orthologs of *OsDro1* have been predicted in *Zea mays*, but their effect on root phenotype were previously unknown. Here we show that mutations in *ZmDro* orthologs lead to significant phenotypic differences, confirming they are involved in root growth in maize as well. In addition, since root architecture is highly variable across the genus *Zea*, we hypothesized that *ZmDro* genes could play a role in modulating quantitative root phenotypes. We sequenced *ZmDro* orthologs across a diverse *Zea* panel including the NAM founder lines, a broad geographic sampling of landraces, and multiple teosinte species. We excavated roots of our panel at five weeks after germination and used 2D imaging and feature extraction to quantify root architecture for comparisons with sequence- and expression-level variation. Currently, we are exploring the potential connection between known phenotypic variation of crown root architecture and molecular level variation in the *ZmDro* orthologs using data from HapMap 3. Determining the link between genetic variation in *ZmDro* orthologs and root architecture across *Zea* will provide further insight into the below-ground impacts of domestication and crop improvement and may ultimately help plant breeders select for an ideal root architecture.

Funding acknowledgement: National Science Foundation (NSF)

P298 

## Field-based measurement of pulling force accelerates the identification of loci controlling root system architecture in maize

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An uncertain climate, paired with rapid human population growth, presents a major challenge to maintaining food security in the twenty-first century. Root system traits represent intriguing potential targets for yield gain and improved stress resilience in cereals. However, we need a greater understanding of the genetics underlying root system architecture (RSA). Additionally, accounting for the effects of genotype-by-environment interactions on RSA is required. Increased throughput of field-based RSA phenotyping technologies will help to facilitate gene discovery. We have developed a high-throughput phenotyping platform based on the measurement of root pulling force (RPF) as a proxy for RSA traits. We used this assay to measure RPF at two different field sites under well-watered and water-stressed conditions across a maize diversity panel. Using GWAS, we have identified candidate loci for RPF on chromosomes 5 and 8. We will present approaches to functionally characterize the roles of these candidate genes on RSA.

Funding acknowledgement: Department of Energy (DOE)

P299

## Fine mapping and transcriptomic dissection of a major genetic locus governing cuticular hydrocarbon chain length on maize silks

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The silk cuticular lipid metabolome includes at least 50 metabolites that are primarily linear hydrocarbons, fatty acids, and aldehydes ranging in chain lengths from 16 to 35 carbon atoms. To identify the genomic loci controlling the biosynthesis and accumulation of these metabolites, we performed metabolite-quantitative trait locus (mQTL) mapping across three years using the intermated B73xMo17 recombinant inbred line (IBMRIL) population, which harbors considerable variation in the silk cuticular lipid metabolome. mQTL analysis of constituent traits, metabolite-class traits, and relative composition traits identified >500 mQTLs that modulate the abundance and composition of the silk cuticular lipid metabolome, with a majority of mQTLs detected in more than one year. To connect this genetic network to the predicted biochemical network for surface lipid biosynthesis, identification of causal genetic polymorphisms or the ability to discriminate among competing candidate gene hypotheses is required. Here we report our progress in dissecting a ~7 Mbp interval on chromosome 4 that accounts for >30% of the genetically explainable variance for each of ~10 biochemical traits associated with hydrocarbon chain length, wherein the B73 allele increases the accumulation of hydrocarbons with chain lengths of 21 to 25 carbons. Two primary candidate genes residing within the locus encode a ketoacyl-CoA synthase involved in elongation of acyl-CoA molecules (hydrocarbon biosynthesis precursors) and a putative fatty acid reductase that converts the acyl-CoA molecules to aldehydes (hydrocarbon biosynthesis intermediates). Both isogenic dual testcross and heterozygous inbred family analyses were used to interrogate critically informative recombination events, narrowing the region of interest to ~2 Mbp and 65 genes. The mapping and fine mapping results are combined with findings from transcriptomic analysis of silks in the parental lines to evaluate the relative likelihood of multiple functional polymorphisms accounting for the observed effects on cuticular hydrocarbon carbon chain length.

Funding acknowledgement: National Science Foundation (NSF)

**P300**

### **From traits to targets -- identifying genes underlying key agronomic traits in a maize structured population**

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Crop improvement through biotechnology requires the introduction of specific genetic changes. In an industrial crop improvement program, the candidate genes and modifications tested are hypothesized to confer a desirable phenotype. While there are multiple techniques for identifying candidate genes for transgenic manipulation, the fast rise of genome editing technologies necessitates more detailed knowledge of the impact of crop genes on phenotypes. The linkage of trait phenotypes to genes within the target crop becomes essential for the full realization of the potential of genome editing. Here, the creation of a genetic population useful for linking specific phenotypes to genes will be described, along with proof of concept data that highlights the utility of this population.

**P301** 

### **Functional basis for hybrid vigor in maize: directional and polygenic effects on yield, height and flowering time**

(submitted by Guillaume Ramstein <[gr226@cornell.edu](mailto:gr226@cornell.edu)>)

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Heterosis has been key to genetic improvement of maize but describing its genetic basis by molecular markers has been challenging. Previous studies of heterosis based on markers have shown significant contribution of dominance effects on heterosis. However, they have generally considered panels of limited genetic diversity and have shown little practical benefit of dominance for capturing or predicting genetic variability in breeding populations. This study aims at characterizing the genetic basis of heterosis in maize for three agronomic traits: days to silking (DTS), plant height (PH) and grain yield (GY). We based our analyses on a diverse panel of temperate lines crossed with two different testers representative of heterotic groups in the United States. First, we confirmed the polygenic nature of dominance effects in the diverse panel for all traits and identified directional dominance effects acting on PH and GY. Then, we identified oligogenic genetic effects influencing hybrid vigor, but only for additive actions on DTS. Finally, we identified significant enrichment of polygenic effects in classes defined by genic regions, structural features (e.g., chromatin openness) and evolutionary features (e.g., GERP scores), especially for PH. Inferences in the diverse panel were validated by concordant associations as well as significant gains in genomic prediction accuracy in a nested association mapping panel consisting of a collection of biparental populations crossed to a single tester. Our results suggest the relevance of polygenic dominance effects and the usefulness of their partition in genic regions for explaining and predicting heterosis in maize breeding populations.

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



P302 

## Genetic analysis of natural variation for photosystem II phenotypes at the leaf level in sweet corn

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Recent advances in quantitative genetic approaches combined with next-generation sequencing and field-based high-throughput phenotyping tools could help to provide insight into the genetic basis of natural variation for the response of photosynthesis in  $C_4$  plants to the local growing environment. We performed a genome-wide association study (GWAS) of six fluorescence-based parameters ( $\Phi_{II}$ ,  $NPQ_{(T)}$ ,  $\Phi_{NO}$ , LEF,  $q_L$ ,  $F'_v/F'_m$ ) associated with photosystem II (PSII) activity in a sweet corn inbred association panel that was genotyped with ~223,000 SNP markers. The MultispeQ v1.0 was used to measure the six PSII phenotypes on the primary ear leaf of sweet corn plants at flowering in replicated field trials in 2017 and 2018. Moderately high broad-sense heritabilities were estimated for all six phenotypes, with the presence of strong genotype-by-year effects. Through the implementation of univariate and multivariate GWAS, a total of 68 unique SNPs was identified to be significantly associated with one or more of the six phenotypes at a genome-wide false-discovery rate of 5%. Notably, a candidate gene encoding a protein predicted to be chloroplast localized and involved in the first step of  $C_4$  photosynthesis underpinned one of the detected GWAS signals. Future efforts will focus on a combination of gene expression and mutagenesis analyses to determine the role of this and other identified candidate genes in the genetic control of PSII phenotypes.

Funding acknowledgement: National Science Foundation (NSF), Hatch, Rural Development Administration, Republic of Korea

P303

## Genetic and bioinformatic investigation of an important genetic factor influencing hydrocarbon accumulation on maize silks

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Very long chain fatty acids, aldehydes, and hydrocarbons are biorenewable chemicals of interest. These compounds constitute the majority of extracellular surface lipids (SLs) on plant surfaces, however the genetic and biochemical pathways responsible for their biosynthesis remain elusive. This project uses maize silks as a model to identify the genes and define the biochemical steps required for surface lipid production. SLs are hypothesized to play a role in protection against biotic and abiotic stresses (e.g. UV radiation, insect damage, and desiccation). During the critical period when silks are exposed for pollination, they face numerous environmental stresses. Our previous metabolite-quantitative trait locus (mQTL) mapping experiments have identified >500 mQTLs residing at approximately 60 genomic locations wherein one or more functional polymorphisms reside and affect whole suites of related surface lipid traits. Here we report a detailed characterization of one of these loci, which is located on the long arm of chromosome 3 and has been implicated as an mQTL for numerous traits, including 9 metabolites (e.g. 25-carbon alkane) and 6 classes (e.g. alkenes). For the class trait “total hydrocarbons”, this locus explains >13% of the phenotypic variance. Based on the phenotypes affected, we infer that one or several coincidentally-located functional polymorphisms are acting to increase flux through the very long chain fatty acid biosynthesis pathway, which would enrich the pool of precursor molecules that can be converted into the many hydrocarbon metabolites whose abundance is affected. Parental backcross and heterozygous inbred families have been created and analyzed for finer scale genetic dissection of this locus, narrowing the list of genetically-derived candidate genes. Bioinformatic approaches to enrich for likely candidate genes has led to the hypothesis that variation in a long-chain acyl-CoA synthase gene likely accounts for the observed phenotypic effects. Results of both the genetic and bioinformatic investigations will be discussed.

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



P304 

### Genetic and functional genomic analysis of tocochromanol levels in maize grain

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Tocochromanols are a family of antioxidants important not only in plant fitness, but also in human health in the form of vitamin E. In this study, we leveraged the tremendous allelic diversity captured in the maize Ames panel of nearly 2,000 inbred lines to identify genes that contribute to the synthesis, transport, and accumulation of tocochromanols in maize grain. We performed a genome-wide association study of nine tocochromanols traits across the Ames panel to identify loci important to the genetic control of natural variation for tocochromanol levels in maize grain. A total of 500 SNP markers was significantly associated with at least one of the nine tocochromanols traits at a genome-wide FDR level of 5%. In agreement with prior studies, an association was detected for *vte4* ( $\gamma$ -tocopherol methyltransferase) and  $\alpha$ -tocopherol, as well as *vte1* (tocopherol cyclase), *hgg1* (homogentisate geranylgeranyltransferase) and *hppd1* (4-hydroxyphenylpyruvate dioxygenase) for tocotrienols. In addition, two chlorophyll biosynthesis genes, *por1* and *por2* (protochlorophyllide reductases), recently reported to explain the majority of variation for tocopherol grain levels in the U.S. maize nested association mapping (NAM) population, were also significantly associated with tocopherol levels in the Ames panel. To study the function of these two chlorophyll biosynthesis genes related to tocopherol synthesis in the non-photosynthetic maize grain, we designed a light/dark treatment experiment on developing kernels of NAM founders and generated multiple mutations at *por1* and *por2* through CRISPR/Cas9 mediated genome editing. The analysis of results from these ongoing functional genomic studies will be presented and connected to our efforts to genetically improve vitamin E levels in maize grain.

Gene / Gene Models described: *vte4*, *vte1*, *hgg1*, *hppd1*, *por1*, *por2*; Zm00001d017746, Zm00001d015985, Zm00001d046558, Zm00001d015356, Zm00001d032576, Zm00001d013937

Funding acknowledgement: National Science Foundation (NSF)

P305

### Genetic architecture of DH and haploid populations in maize: its similarity and specificity

(submitted by Jinlong Li <[734316892@qq.com](mailto:734316892@qq.com)>)

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Ploidy variation is an important reason of genetic evolution in nature. With the help of doubled haploid (DH) technology, materials with different ploidy level is generated, which offers an opportunity understand the genetic bases of ploidy evolution. Here we constructed a DH population and its corresponding haploid population to study the genetic architecture of ploidy variation. DH lines from C7-2×Qi319 were obtained by doubled haploid technique, and then DH lines were crossed by haploid inducer to obtain corresponding haploid lines. Then the genetic structure of ploidy effect was studied by haploid and diploid population. We collected the 15 agronomic traits of the 2 populations at 2 locations in 2 years. The QTL mapping results showed that the loci conferring to plant height in haploid and DH population was 2 and 1, respectively. In addition, one locus overlapped at chromosome 1. 3 loci were detected in haploid population and 1 locus in DH population, among which 1 locus is co-located. These results show that although the genotype is identical in haploid and diploid population, there was different genetic structure between them. According to our results, haploid population can be used to find new locus that cannot be detected in diploid population.

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P306 

## Genetic resources to explore functional diversity in Mexican native maize and teosinte

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Mexican maize landraces and their teosinte wild-relatives represent a unique source of variation: genetically, they are more variable than modern breeding lines; at the phenotypic level, they are morphologically diverse, and show broad adaptation, often remaining productive in marginal or low-input systems. The increasing availability of genomic data has allowed identification of candidate genomic regions and sequence variants linked to local adaptation, specific environmental variables, and morphological and agronomic traits. Functional validation of such candidates, however, can be difficult: large differences in phenology and adaptation present logistic and methodological challenges to *per se* evaluation; the trait of interest can be masked by background variation; the heterogeneous nature of outbred material makes it hard to estimate genotypic values. To address these difficulties, the Mexican National Laboratory of Genomics for Biodiversity (LANGEBIO) is developing a range of genetic resources derived from crosses between landrace/teosinte diversity and modern inbreds, including bi-parental recombinant inbred lines, introgression lines, and multi-parent populations. Here, we will present advances in the generation, genetic analysis and use of this material. We invite inquiries from any colleagues interested in exploring the application of these resources in their own work.

Funding acknowledgement: National Science Foundation (NSF), CONACYT (MEXICO)

P307 

## Genome assembly of five Stiff Stalk inbreds: PHJ40, LH145, PHB47, NKH8431, & B84

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Our team has a goal of developing and acquiring resources to support maize research in elite genetic backgrounds that are efficient research tools and that facilitate technology transfer. The Stiff Stalk heterotic pool is a cornerstone of commercial U.S. cornbelt dent hybrids. B73 – the inbred chosen for the current reference genome – is an influential founder in this group. B14, B37, and B84 are additional influential Stiff Stalk Synthetic founder inbreds, and companies have internally developed additional lines within this heterotic group. We constructed whole-genome reference-guided assemblies of five Stiff Stalk ex-PVP and public inbred lines. LH145 represents the B14 founder group, and PHB47 represents the B37 founder group. B84 is found in multiple commercial pedigrees and is related to the B73 group. PHJ40 is an early Stiff Stalk type developed by Pioneer Hi-Bred. NKH8431 was Northup King’s recombination of B73 and B14 based germplasm for early maturity. Genomes were sequenced using PacBio, assembled using MECAT, polished using QUIVER, and pseudochromosomes were generated using reference-guided assembly relative to the B73 version 4 genome. Misjoins were mitigated by breaking and reassembling the sequences, and Illumina sequencing was used to correct SNPs and indels. The project is being finalized, but example assembly statistics for PHB47 and PHJ40 exemplify results of the project. The PHB47 genome was assembled into 3841 contigs and 940 scaffolds, resulting in 2155.6 Mb of assembled sequence space. The PHJ40 genome was assembled into 1547 scaffolds consisting of 4250 contigs, resulting in 2153.8 Mb of assembled sequence. Annotation supports that >96% of the B73 version 4 annotated primary transcripts are present in these assemblies. Details on the sequencing process and outcomes will be described. In addition, the genetic relationships among inbreds will be presented and the association with phenological information will be described to support their utilization in research programs.

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

P308 

## Genome wide analysis of *Ga1-s* modifiers in maize

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A one way reproductive barrier is present between most popcorn varieties and most dent corn varieties grown in the temperate USA. This barrier is predominantly controlled by the *gal* locus, which prevents dent corn pollen from successfully fertilizing popcorn ears. Using data from a diverse population of popcorn accessions pollinated by a dent corn tester, we found that the non-reciprocal pollination barrier conferred by the *Ga1-s* allele is more complex than previously described. Individual accessions ranged from 0% to 100% compatible with dent corn pollen. Seven significant modifiers of dent pollen compatibility were identified on five chromosomes. The existence of *Ga1* modifiers segregating in a native popcorn background may indicate selective pressure to allow gene flow between populations, which should be incorporated into future models of the impact of genetic incompatibility loci on gene flow in natural and agricultural plant populations.

P309 

## Genome wide association study for resistance to foliar diseases in corn lines

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The identification of genotypes resistant to foliar diseases using phenotypic selection is not efficient, and therefore more effective methods for the identification of these genotypes, such as marker-assisted selection, has been recommended. The objective of this work is to identify causal genomic regions associated with resistance to foliar diseases as a useful tool in maize breeding. For this, a broad genomic association study was conducted to identify significant associations between the markers and possible causal regions related to resistance to the following leaf diseases: Gray leaf spot - *Cercospora zea-maydis*; Northern corn leaf blight - *Exserohilum turcicum*; Maize white spot - *Phaeosphaeria maydis*. Thus, an experiment with 230 diverse inbred lines was planted in 4 environments under natural epidemics. The phenotypes were measured according to a rating scale for each disease. The inbred lines were genotyped with 23153 DArT markers. The association analysis was carried with different mixed models, where we evaluated the effects of the incorporation of the degree of kinship (K matrix), as well as use of population structure, via main principal components (PC). Initially the associative mapping analyzes were performed separately for each feature evaluated through the Gapit and BGRL package. High marker effects and significant regions were found for these diseases in maize chromosomes 1,2,4,6,8 and 10. In addition, we calculated the phenotype prediction accuracies of genomic selection models generated using the same dataset. Future steps include the fine mapping and validation of the associations. We expect to use these results in the development of functional markers for marker assisted selection as well deploying genomic selection for disease resistance. Altogether, these results could accelerate the breeding process and increase the efficiency of selecting resistant inbred lines. This text is not italicized. *This text is italicized.* This text is not italicized.

Funding acknowledgement: United States Department of Agriculture (USDA), Coordination for the Improvement of Higher Education Personnel (CAPES)

P310 

## Genome-wide association analyses of maize kernel traits in the Wisconsin diversity (WiDiv) panel

(submitted by Barbara Muller <[mullerbsf@ufl.edu](mailto:mullerbsf@ufl.edu)>)

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Kernel composition and density are important traits for nutritional quality and kernel hardness in maize. To investigate genetic variation controlling these traits, single-kernel near infrared reflectance (NIR) spectroscopy was used to phenotype 400 maize inbred lines from the Wisconsin diversity (WiDiv) panel. The NIR platform collects individual kernel weight, total density, total volume, material density, material volume, percent oil, percent protein and percent starch. We carried out genome-wide association studies (GWAS) for these eight traits with single-SNP, gene and segment-based models, as well as regional heritability mapping using 605,384 polymorphic SNPs from published RNA-seq genotypes. The traits showed a wide range of genomic heritability ( $h_g^2$ ) from 0.24 for material density to 0.98 for percent oil. The number of significant associations detected was roughly correlated to heritability. The largest number of significant associations were detected for percent starch ( $h_g^2=0.63$ ) and oil ( $h_g^2=0.98$ ). With gene-based GWAS, five genes were significantly associated with percent oil on chromosomes 6 and 9. Importantly, we identified the diacylglycerol acyltransferase at the *linoleic acid1 (ln1)* locus as a major contributor to oil content. Regional heritability mapping of percent oil with 12,093 overlapping windows of 100 SNPs each identified regions of chromosome 6 and 9 as explaining 47% and 22% of the phenotypic variance, respectively. Our results illustrate that GWAS is most effective in identifying genome segments for traits with simpler genetic architecture. This study further corroborates the complex architecture of some kernel traits and suggests that considerably larger samples and/or improving the accuracy of phenotyping methodologies will be necessary to capture any relevant portion of the genetic variance for maize kernel traits.

Funding acknowledgement: National Science Foundation (NSF)

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## Genome-wide association studies of B vitamin levels in maize grain using 16.7 million imputed SNPs

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Maize-based diets are low in B vitamins, which are essential nutrients. Chronic deficiencies in B vitamins remain common, especially among women and children in developing countries. This collaborative project aims to identify the genetic basis of B vitamin content in maize through genome-wide association studies (GWAS). During 2015 and 2017, approximately 2,000 inbred lines from the Ames Panel were grown and B vitamin content determined using HPLC. The B vitamin traits studied were thiamine, thiamine (13C), and thiamine (d3) (B1 traits); riboflavin (B2); nicotinic acid, nicotinamide, trigonelline, and niacin (B3 traits); and pyridoxic acid, pyridoxine, pyridoxamine, pyridoxal, B6, and B6 without pyridoxal (B6 traits). Genome-wide scans were conducted on each of these traits with approximately 400,000 SNPs using four GWAS methods: mixed linear model, multi-locus mixed model, FarmCPU, and QTCAT; results of these methods were compared. This analysis detected significant associations for eleven of the fourteen B vitamin traits analyzed. In addition, resequencing data for the 277 lines of the Maize Association Panel available from Panzea were used to impute high-density genotypes for the Ames Panel lines. Beagle was used for imputation (mean imputation accuracy 91.9%). The final imputed SNP dataset contained 16.7 million markers, which was used to conduct mixed linear model GWAS. Using this high-density SNP dataset revealed more and stronger associations for many of the B vitamin traits, including significant peaks for one of the traits that had no significant associations detected using only 400,000 SNPs. All of these results appear to be novel loci when compared to a candidate gene list from *Arabidopsis*. Significant associations are being investigated, and selected genes will be validated with CRISPR mutagenesis. Understanding gained from this project will be used in selection and breeding of maize for improved B vitamin content.

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## Genome-wide association study of mineral levels in maize grain reveals candidate genes for mineral uptake and transport

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Collectively impacting more than two billion people worldwide, mineral nutrient deficiencies, principally in iron (Fe) and zinc (Zn), are global health problems that predominantly occur in the developing world. The maize Ames inbred panel, with a population size of nearly 2,000 lines and capturing a high level of allelic diversity, provides a powerful platform for studying the genetic basis of mineral concentration in maize grain. Inductively coupled plasma-mass spectrometry was used to generate grain mineral profiles consisting of 11 elements (B, Ca, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Zn) for Ames inbred lines grown at a single location (Indiana, USA) with an augmented incomplete block design in 2012 and 2013. Most mineral traits were found to have a strong genetic component, with broad-sense heritabilities ranging from 0.42 for B to 0.86 for Cu, suggesting that these traits should be amenable to genetic dissection. A genome-wide association study was conducted with ~390,000 SNP markers scored on the Ames panel to identify genes underlying the genetic architecture of these 11 traits. A total of 157 SNP markers were significant for one or more mineral traits at a genome-wide FDR of 10%. The open reading frame of eight candidate genes having a likely associated role in mineral uptake and transport were found to contain at least one SNP that significantly associated with one of six (B, Cu, Mn, Mo, Ni, Zn) mineral traits. With supporting genetic information from a joint-linkage quantitative trait loci analysis of the same traits in the U.S. maize NAM population, an additional candidate gene that likely plays a key role in Zn transportation was identified on chromosome 7. The knowledge generated from this study will ultimately help to accelerate genomics-assisted breeding efforts for improved grain mineral concentration in maize biofortification breeding programs around the world.

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## Genome-wide association study reveals the genetic architecture of leaf cuticular evaporation rate in maize

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The cuticle, a hydrophobic layer of cutin and waxes synthesized by plant epidermal cells, limits excess water loss and protects plants against pathogen attack. While cuticular evaporation (CE) accounts for about 5-10% of water loss in well-watered plants during the day, it is the major source of water loss when stomata are closed at night and under water-limited conditions. Elucidating the genetic basis of CE rate is important for understanding the cuticle biosynthesis pathway and improving crop adaptation to drought-prone environments. In that light, we conducted a genome-wide association study of CE rate across a maize inbred association panel that was evaluated in four environments (Maricopa, AZ, and San Diego, CA in 2016 and 2017) and genotyped with ~250,000 single-nucleotide polymorphism markers. The broad-sense heritabilities of CE rate ranged from 0.44 to 0.71 within locations and across all environments, and CE rate had a very weak correlation with flowering time in all environments. Significant GWAS signals were identified for CE rate on chromosomes 1 and 4 (short arm) for the Maricopa location and on chromosomes 4 (long arm) and 10 for the San Diego location. Additional signals associated with CE rate were found on chromosomes 2, 5 and 10 across all environments. The signals were underpinned by candidate genes involved in cuticle biosynthesis and deposition, as well as intracellular trafficking and cross membrane transport of cuticular waxes. The findings of this study provide novel insights into the genetic architecture of CE rate and have the potential to help breeders more effectively develop drought-tolerant maize for target environments.

Funding acknowledgement: National Science Foundation (NSF)



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### Genotype imputation from Maize Association Panel to Ames Panel

(submitted by Mahule Elyse Boris Alladassi <[aboris@iastate.edu](mailto:aboris@iastate.edu)>)

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The recent advances in next-generation sequencing have significantly increased the number of lines that are re-sequenced for variant discovery. Even with reduced costs, re-sequencing a large number of lines and variants necessary for identifying important trait associations in Genome-Wide Association Studies (GWAS) is still not feasible with current research budgets. Genotype imputation makes it possible to obtain high-density genetic markers for a large number of samples with a fraction of the cost. Recently, Panzea released 20 million SNPs from re-sequencing the 277 lines of the Maize Association Panel. We hypothesized that these high-density SNPs can be leveraged to increase the power for detecting genetic signals from the Ames Panel that has a large sample size but low-density SNP coverage. The BEAGLE haplotype-frequency model was used to build the reference haplotypes from 277 lines with 20 million SNPs. Next, 371,000 anchor SNPs were used to impute the 20 million SNPs to the 2,812 lines of the Ames Panel. Genome scans of multiple traits were conducted to compare the results from low and high-density SNP datasets. Our results suggest that leveraging high-density SNPs significantly increases the power to detect genetic signals in GWAS.

Funding acknowledgement: National Science Foundation (NSF), Iowa State University Raymond F. Baker Center for Plant Breeding, Iowa State University Plant Sciences Institute

P315

### Hayman's diallel analysis for studying the inheritance of aluminum tolerance in tropical popcorn

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Understanding the genetic control of root growth is essential to improve aluminum (Al) tolerance in crops. The objective of this study was to investigate the inheritance of Al tolerance of tropical popcorn. Eight inbred lines were crossed in a diallel design and jointly assessed with their F1 hybrids and two tolerant checks in a completely randomized design with two replications. The Al tolerance related traits were relative and net total, lateral, and axial root length and number of root tips. The diallel analysis followed the Hayman's proposal. For all traits we observed adequacy of the Hayman's six hypothesis, i.e, homogeneity of  $W_r - V_r$  and regression of  $W_r$  on  $V_r$  with slope 1. The coefficient of determination ranged from 66 to 92%. There were genetic variability and dominance for all traits. Only for net axial root length there was bidirectional dominance. The dominant genes are not symmetric distributed between the parents. The dominance was negative for relative total, lateral, and axial root length and positive for the other three traits. In general, there was evidence of complete dominance. Only for relative lateral root length and net axial root length there were overdominance. None of the assessed inbred lines has reached the selection limits. The inbred lines 11-133, 11-403, and 11-383 have the highest number of recessive (favorable) genes for relative total, lateral, and axial root length, respectively. Regarding net total and lateral root length and root tips number, the inbred line 11-142 has the highest number of dominant (favorable) genes.

Funding acknowledgement: National Council for Scientific and Technological Development (CNPq), the Brazilian Federal Agency for Support and Evaluation of Graduate Education (Capes)

P316 

## High-throughput phenotyping of maize root system size and distribution in the field through data extraction from minirhizotron images using machine learning

(submitted by Andrew Leakey <[leakey@illinois.edu](mailto:leakey@illinois.edu)>)

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The major goals of the broader project are to identify the genetic basis of maize root architecture and its relationship to soil nitrogen availability and plant nitrogen status. This poster describes the development and testing of methods to perform high-throughput phenotyping of root system size and distribution in field-grown mapping populations of inbred and hybrid maize. Previous methods for large-scale phenotyping of maize roots were limited to controlled environment conditions or root crowns of field grown plants. Minirhizotron technology allows imaging of roots across the full soil profile in the field. But, throughput of image acquisition and image analysis needed to be increased by two orders of magnitude over standard methodologies. In 2017 we used our modified tractors and pneumatic coring equipment to install >1500 minirhizotrons, and collected ~120,000 images of root size and distribution. We have built a pipeline to automatically analyze those images. It has been used to generate root length density profiles to a depth of 1 m for ~300 RILs from the Illinois Protein Strain Recombinant Inbred (IPSRI) population in 2017. Approximately 100,000 images analyzed by hand were used to train and validate the predictions of the machine learning analysis pipeline. We see significant genetic variation in root size, depth and distribution within our mapping population. We are currently assessing how root traits estimated from other phenotyping approaches correlate with the minirhizotron derived data and preparing to analyse genotype to phenotype associations. In 2018, we have again doubled the scale of the experiment and have installed ~3000 minirhizotrons in ~500 hybrid genotypes. We collected ~300,000 images of root length and distribution in the field, and are currently evaluating genetic variation in root system size and distribution. These experiments demonstrate the capability to phenotype root depth distribution in the field at a scale that is meaningful for quantitative genetic analyses. In the future, we hope to combine knowledge gained through these experiments with phenotypic data gathered with other methods to understand the genetic basis of root architecture and limiting N conditions.

Funding acknowledgement: National Science Foundation (NSF)

P317 

## How water limitation affects $\delta^{13}\text{C}$ , a proxy trait for water-use efficiency in maize

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Maize is a major staple food, feed, and bioenergy crop that is cultivated across the globe. Climate change is projected to alter normal rainfall patterns which will result in more frequent water limiting conditions. A challenge facing researchers today is to develop crops that are better able to meet these changes given that the majority of food is produced via rainfed agriculture. One method to improve the resiliency of crops towards climate change is to increase their Water-Use Efficiency (WUE). However, WUE is a challenging trait to quantify, often requiring specialized equipment or labor intensive greenhouse experiments. It has been shown that the leaf stable carbon isotope ratio,  $\delta^{13}\text{C}$ , is a proxy trait for WUE in maize. The effect of drought on  $\delta^{13}\text{C}$  has been well described in  $\text{C}_3$  species and to a lesser degree in  $\text{C}_4$  species. Understanding how water availability influences  $\delta^{13}\text{C}$  can better allow researchers to utilize this measurement. Our preliminary experiment showed that  $\delta^{13}\text{C}$  has high correlations with traits such as biomass and transpiration, and thus, agronomic WUE. This result suggests potential applications for the use of  $\delta^{13}\text{C}$  in breeding. A water-treatment trial revealed that  $\delta^{13}\text{C}$  closely follows the amount of water that is transpired, and a developmental time-course study showed that  $\delta^{13}\text{C}$  is gradually and cumulatively affected by water limitation. Furthermore, the change in  $\delta^{13}\text{C}$  can be observed in the first emerged leaf after a water limitation is imposed. This highlights the need for accurate sample timing when studying  $\delta^{13}\text{C}$ . A Genome Wide Association Study meant to identify the genetic control of leaf  $\delta^{13}\text{C}$  did not find any significant SNPs, but did identify regions that had significant associations with the C/N ratio of leaf tissue. Understanding the physiological and genetic components underlying  $\delta^{13}\text{C}$  will facilitate the future optimization of whole plant WUE.

Funding acknowledgement: United States Department of Agriculture (USDA)

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### Identification of inbred-specific modifiers for *narrow odd dwarf (nod)*

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The *narrow odd dwarf (nod)* EMS mutant has pleiotropic developmental defects that vary greatly across different genetic backgrounds. Homozygous *nod* mutants in the B73 background are dwarf, with short and narrow leaves, increased tillering, and disrupted floral development. Homozygous *nod* mutants in the Mo17 background, however, are nearly indistinguishable from wild-type Mo17 plants. The A619 *nod* mutant phenotype falls between these two extremes. To identify modifying loci in these genetic backgrounds, we crossed homozygous *nod* mutants in A619 and Mo17 to heterozygous *nod* mutants in B73, and then self-pollinated homozygous *nod* individuals in the F1 population to generate F2 populations segregating for B73, A619, and Mo17 modifiers. We scored the F2 population for several phenotypes, including plant height, leaf width, and leaf length. These data were normally distributed across the population, indicating that multiple modifiers affect the *nod* phenotype. We extracted DNA from a random subset of individuals from these populations for genotyping-by-sequencing (tGBS®) to identify loci that affect expression of the *nod* mutation in different inbred backgrounds.

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

P319 

### Identification of quantitative trait loci effective against bacterial leaf streak of maize

(submitted by Yuting Qiu <[yutingq2@illinois.edu](mailto:yutingq2@illinois.edu)>)

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Bacterial leaf streak (BLS) of maize is a bacterial foliar disease caused by *Xanthomonas vasicola* pv. *vasculorum*. BLS recently emerged in the United States and has reached epidemic levels in the western corn belt. Currently, little is known about management of the disease, and no chemical control is available. Host resistance will play a role in managing the disease, but no studies have been published to date on the genetic architecture of resistance to BLS. We conducted quantitative trait loci (QTL) mapping in three populations: DRIL78, NAM Z022, and NAM Z023. DRIL78 is a disease resistance introgression line (DRIL) population with NC344 and Oh7B as parents; Z022 and Z023 are nested association mapping (NAM) populations with B73 and Oh43 and B73 and Oh7B as parents, respectively. Here we present our results for the QTL mapping. We found five significant QTLs related to BLS resistance across chromosomes 1-5. Two regions were involved in resistance to other maize diseases. Overall, our results provide insight to the genetic architecture of resistance to BLS and lay the foundation for breeding BLS resistant lines.

Funding acknowledgement: FFAR

P320

## Identifying and parameterizing corn leaf and canopy characteristic for crop modeling

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Traditional breeding is based on observation of phenotypic traits to select superior genotypes. More recently, genomic selection has enabled selection based on genetic data alone, after training a model to relate genotypic to phenotypic variation. However, plant performance not only depends on the genotype, but is also affected by the environment and genotype-environment interactions, which decrease the accuracy of both phenotypic and genomic selection. Improving predictions of plant performance in diverse environments would be useful for selecting proper varieties, from both a breeding and production standpoint. One approach to improving these predictions is to leverage physiological crop growth models to incorporate environmental factors and their impact on complex traits. To make this useful for breeders, models will need to be parameterized on a genotype-specific basis. The objectives of the current study are to (i) identify heritable leaf and canopy characteristics that can be used as model parameters, (ii) measure and model these parameters across diverse maize lines, (iii) estimate the accuracy of prediction for the model parameters. The power function of leaf area distribution calculated from ear leaf area was used to calculate total leaf area, and the estimates for leaf 6 were compared to observed values. These traits will be used alongside canopy characteristics as the parameters for physiological crop growth models to accurately predict high-level complex traits. Future work will include analysis of multi-environment phenotypic data and genomic prediction to improve prediction of new genotypes in unobserved environments.

P321 

## Identifying genes that regulate panicle architecture of sorghum: GWAS coupled with semi-automated, high-throughput image analysis

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The manual collection of inflorescence phenotypes can be time-consuming for the large populations needed to conduct GWAS (genome-wide association studies) and is difficult for multi-dimensional traits such as volume. A semi-automated phenotyping pipeline (Toolkit for Inflorescence Measurement, TIM) was developed and used to extract uni- and multi-dimensional features from images of 1,064 sorghum panicles from 272 genotypes comprising a subset of the Sorghum Association Panel (SAP). GWAS detected 35 unique SNPs associated with variation in inflorescence architecture. The accuracy of the TIM pipeline is supported by the fact that several of these trait-associated SNPs (TASs) are located within chromosomal regions associated with similar traits in previously published QTL and GWAS analyses of sorghum. Additionally, sorghum homologs of maize and rice genes known to affect inflorescence architecture are enriched in the vicinities of TASs. Finally, our TASs are enriched within genomic regions that exhibit high levels of divergence between converted tropical lines and cultivars, consistent with the hypothesis that these chromosomal intervals were targets of selection during modern breeding.

Funding acknowledgement: United States Department of Agriculture (USDA)

P322 

## Image-based phenotypic platform for monitoring maize growth to estimate end-season productivity

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Large progress has been made over the last decade linking genomic information to phenotypic information and using this to enhance crop productivity. However, further progress in this area requires a better understanding of how genetic and phenotypic elements interact with the environment throughout development. In order to capture plant responses through time robust, rapid and low cost methods to evaluate plants growing in fields need to be implemented. Unmanned aerial systems can be an efficient means to do so. We have developed a procedure for utilizing RGB drone imagery to extract phenotypic traits including stand count, plant height, canopy closure, plant rotation and growth rate, and have used this pipeline to evaluate how plants change and develop in response to the environment they are grown in and how this correlates with end season yield in maize. This platform was used to characterize a yield trial consisting of 24 maize hybrids planted in replicate under two dates (mid May and late May) and three densities (60k, 90k and 120k plants per hectare) in St Paul, MN in the summer of 2018. The field was imaged weekly after planting using a DJI Phantom 4 Advanced drone and traits were extracted following data collection. Ear and seed morphological characteristics and yield were collected at the end of the season. Significant temporal phenotypic responses were observed across the hybrid entries in response to the suite of environments. Results on accuracy of trait extraction algorithms compared to manual measurements and on the utility of temporal trait data for predicting end season yield in maize will be presented. Monitoring crop growth can be used to assess resiliency of lines across different environments and implemented in the long term to carry out informed management decisions that reduce yield penalties in an environmentally sustainable way.

P323 

## In-field whole plant architecture characterized by scalable, affordable robots

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Recent advances in computational phenotyping are driving a revolution in how plant traits are defined and measured. Affordable sensors and computational hardware enable higher accuracy, precision, scale, and throughput than traditional phenotyping methods. In this study, we used an affordable 2-dimensional light detection and ranging (LIDAR) device mounted on an automated, 30 cm tall, sub-canopy EarthSense rover (TerraSentia) to generate 3-dimensional reconstructions of plant architecture. The LIDAR data were collected in a Genomes to Fields maize hybrid trial in Aurora, NY, resulting in billions of data points representing 714 hybrids partially replicated in 1083 two-row plots. Traditional architectural traits such as plant height can be predicted with accuracy >0.75 using LIDAR data alone relative to manual measurements, demonstrating the effective capture of plant architecture by this method. By performing dimensional reduction on the raw LIDAR data, we produced novel, holistic descriptors of plant architecture and biomass distribution. These novel descriptors have heritabilities as high as 0.6, matching or exceeding heritabilities for manually measured architectural traits in the same population. These results set the groundwork for describing plant architecture, development, and GxE in a holistic framework and suggest the feasibility of automated and high-throughput plant architecture phenotyping.

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P324 

## **Inbred yield trial replacing preliminary single-cross hybrid yield trial is not viable in scaled commercial breeding programs**

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There were three researchers – Schnell (1974), Duvick (1999) and Mikel (2008), conducted independent, comprehensive heterosis studies on corn (*Zea mays L.*) based on historical time series spanning from 1916 to 2005. Forrest Troyer (2009) summarized their results and concluded parental inbred and hybrid yield have been increasing simultaneously, while percentage heterosis decreased significantly across time (2.8%/year). Inbred yield increase per breeding cycle was 2.7 times faster than hybrid from 1976 through 2005. Consequently, he put up the concept that yield test finished inbreds to replace preliminary single-cross corn yield test will increase rate of commercial hybrid yield gains, and moreover, cost effective. Correlation studies of inbred and hybrid yields showed an increasing trend from ~0.2 in Hallauer (1988) to 0.36 in Mikel (2008). It was stated that the correlation was not strong enough to be indicative of hybrid yield, thus breeding community has been conducting hybrid yield trial during the past decades. We carried out yield trial of 3,568 inbreds and testcrosses to evaluate the 2 concepts. Results showed that the testcross yield was 1.87 times of inbred yield, and heterosis was 45% of testcross yield, averagely; which was 2.81 and 64% in 1930, 2.02 and 50% in 1980, respectively. A positive correlation coefficient (0.36) was found between inbred (F<sub>4</sub>) and testcross hybrid grain yield. However, based on our testing system, inbred yield trial was \$21/entry more cost than testcross yield trial. Additionally, the low vigor of inbreds aggravated the border effect and distorted the yield data accuracy. Our conclusion is, inbred yield trial replacing preliminary hybrid yield trial is a good concept but not economically viable at this time.

P325

## **Incorporating environmental data to assess the effect of selection on maize performance stability**

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Plant breeders utilize artificial selection to maximize productivity of crops for the set of conditions for which cultivars are targeted for. To ensure dependable performance, breeders assess genotypes across relevant environments. The goal of this research is to assess how selection for performance has influenced trait stability responses and how incorporating climatic data into these analyses can help us improve performance predictability. A set of 102 hybrids was generated by crossing inbred derivatives with varying levels of selection from the maize Iowa Stiff Stalk Synthetic Population by a single tester. These hybrids were evaluated across 31 environments as part of the Genomes to Fields Initiative using a randomized complete block design with two field replications for two years. Agronomic and phenological traits were evaluated for performance stability across environments using two different environmental indices applied to the Finlay-Wilkinson linear regressions. The first index, which has traditionally been used in stability analyses, is performance-based and uses the average cultivar yield in each location. The other is environmentally-based and uses average cultivar photothermal time at flowering for each location. Yields range from 108.5 to 265.5 (bu/A) and photothermal units at flowering range from 14,377 to 22,314 (PTU). Hybrids were analyzed in groups based on the level of selection and the slope and mean squared error (MSE) estimates from both indices suggest an increase in overall stability coincides with improved trait performance. For slope, root and stalk lodging exhibited the greatest decrease in MSE across hybrid groups. Despite common stability trends between indices, the performance-based index slope and MSE estimates tended to be more significantly near 1 and 0, respectively. This suggests that the environmentally-based index could be used to identify a larger range of cultivars exhibiting stability. The continued implementation of climatic data is expected to enhance the predictability of breeding outcomes.

Funding acknowledgement: United States Department of Agriculture (USDA)



P326

## Increased predictive accuracy of maize hybrids modeling environmental correlations in genomic selection

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Genomic selection (GS) has become an important tool to accelerate the selection of superior genotypes and increase genetic gains in breeding programs. However, despite of its advantages, it has often been used only in the initial stages of selection to eliminate individuals with low performance, once the correlations obtained between the predicted and observed values are still low. At this stage, the best strategy to model the environmental effect and the best strategy to model the environment effects is not well defined. Thus, the goal of this study was to evaluate the predictive accuracy of the GBLUP genomic selection model considering the dominance effects and the genotype by environment interaction (GxE). To execute this project, 477 maize lines were genotyped and phenotyped, and 3080 hybrids were phenotypically evaluated for grain yield, in nine seasons and in 55 sites. In all evaluated seasons, the broad sense heritability ranged from 0.31 to 0.67. Most part of the genetic variation was due to the dominance effect and the correlations obtained between the observed and predicted genetic values, when adopting the GBLUP model ignoring the effect of GxE interaction, were low. We observed a high influence of the environment on the behavior of the evaluated genotypes, through estimates of the GxE interaction variance and through the ratio  $\sigma^2_{gxe}/\sigma^2_g$ . The environment effect was considered the main factor responsible for the low correlation obtained. Therefore, the next step of this study will be to model the effect of GxE interaction through a genetic correlation matrix between the environments, aiming at increase the predictive capacity of new hybrids and consequently obtain more accurate selections and in greater genetics gains.

Funding acknowledgement: United States Department of Agriculture (USDA), Coordination of Superior Level Staff Improvement (CAPES)

P327 

## Investigating a cereal killer: Exploring quantitative resistance to the parasite *Striga hermonthica* in sorghum

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Parasitic plants rely on host plants to complete their lifecycle by feeding directly on the host through a haustorial connection. Approximately 1% of angiosperms utilize varying degrees of parasitism for survival. Perhaps the most notable members of the parasitic Orobanchaceae family are the *Striga* species. *Striga* parasitizes key agronomic species resulting in a significant negative impact on crop productivity worldwide. *Striga* species have specific host preferences, and *Striga hermonthica* preferentially parasitizes host plants in the Poaceae family, notably maize, rice, and sorghum. *S. hermonthica* is a hemiparasitic plant that parasitizes sorghum and can result in a complete yield loss. Natural genetic variation for *S. hermonthica* resistance exists in sorghum, but the genetic architecture of this trait is not well understood. The sorghum PP37 multi-parent advanced generation inter-cross (MAGIC) population was established using 25 founder inbred lines, 23 of which are resistant or tolerant to one or more *Striga* species. To understand the genetic architecture of *S. hermonthica* resistance in sorghum, the PP37 MAGIC population comprising 1000 inbred accessions was phenotyped for *S. hermonthica* resistance in Northwestern Ethiopia for two growing seasons. The data from these field trials were used to conduct a genome-wide association study. Here we present the results of this ongoing study and the nuances of conducting effective international plant science research on a highly quantitative trait will be discussed.

Funding acknowledgement: Bill & Melinda Gates Foundation

P328 

### Investigating genetic control of maize kernel morphology

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Component traits such as kernel number, size, and weight are important for determining the contributing factors of yield differences in maize, which has a very high degree of trait variance across genotypes and environmental conditions. Automated morphological analysis of the kernels can help estimate some of these component traits, potentially providing more fine-tuned targets for selection or insight into developmental pathways. To describe variation in kernel morphology, groups of kernels from ~800 different genotypes were first imaged using a modified light box and flatbed scanner. An ImageJ pipeline was modified to isolate the outlines and measure each kernel's area, roundness, aspect ratio, and other traits. Principle component analysis was performed to visualize variation present in the genotypes and determine variance contributions from each trait. After this, a genome wide association study, or GWAS, was performed to identify variants at genomic loci associated with complex traits within the population by finding linkages between single-nucleotide polymorphisms (SNPs) and the traits identified from ImageJ. Further research will be aimed at cross-referencing yield results with the initial kernel data and genomic information to determine which traits and genetic regions influence different aspects of corn development including yield, growth rate, and more.

P329

### Investigating phenotypic effects of cold stress in maize seedlings using image analysis

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Maize is one of the most widely grown crops in the United States, comprising 95% of feed grain. Due to this crop requiring high temperature to germinate and grow, low temperature is a main contributor to shortened growing seasons and low yields. Low temperature reduces carbon fixation, electron transport, and overall negatively impacts plant growth and development. Climate change also plays a role in the need for plant adaptation as it leads to a decrease in arable land. Therefore, discovering genetic factors controlling response to cold stress is crucial for crop improvement. B73 and Mo17 are maize inbreds that served as parental genotypes for many commercial hybrids. B73 shows strong negative response when exposed to cold during the seedling stage, while Mo17 is more tolerant. Studying these lines may reveal what allows maize to grow under colder temperatures. Our goal was to better understand the genetics of cold tolerance in maize and identify phenotypic parameters that benefit quantitative trait loci (QTL) mapping. Using image analysis conducted at the University of Minnesota, we investigated 19 parameters that could be affected by cold stress and examined the relationships between the environmental stress and genotype for those parameters. Images of each plant were taken daily starting eight days after planting. Various parameters, such as plant height, leaf color, leaf area, etc. were extracted from the images. Plants exposed to cold (4C for 8 hours) were compared to plants grown under control conditions. Data was analyzed by a two-way ANOVA test showing parameters with strong environment genotype interactions. These parameters are expected to show differences in response to cold by different inbreds. Our analysis found several parameters that showed significant environment genotype interactions over several days of plant growth, making them strong candidates for QTL analysis and gene mapping.

Funding acknowledgement: National Science Foundation (NSF)

P330 

### **Kernel abortion during haploid induction in maize (*Zea mays* L.)**

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The doubled haploid technology involving in vivo haploid induction is one of the most implemented tools to develop inbred lines in maize breeding. The success of this technology has been due to maize inducer lines development and application, and has become the preferred way of producing maize haploids. However, even though most seeds develop normal endosperm and embryos when using haploid inducers as male parents, a certain proportion of haploids seem to be abnormal (e.g., aborted). Previous results found a relationship between aborted kernels and haploid induction. Moreover, the biological mechanism by which haploid induction and abnormal kernel occurs in maize is still unclear. In this study we carried out an association mapping analysis to verify if there is any region of the maize genome associated with kernel abortion and which genes might potentially be involved. Using a subset of the Ames (Romay et al. 2013) panel with >250 inbred lines induced using a haploid inducer, genotypic data and phenotypic data were collected and a statistical analysis such as ANOVA and Association analysis carried out for the trait of kernel abortion. Preliminary results showed that there was phenotypic variation among different inbred lines for kernel abortion thus indicating that regions of the maize genome and consequently specific genes likely might be involved in causing kernel abortion.

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### **Loss of genetic diversity in doubled-haploid lines from European maize landraces**

(submitted by Leo Zeitler <[leo.zeitler@gmail.com](mailto:leo.zeitler@gmail.com)>)

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Inbreeding depression describes the underperformance of selfed individuals and is particularly strong in predominantly outcrossing plants such as maize. A potential reason for inbreeding depression is the homozygosity of slightly deleterious recessive alleles that are otherwise masked in the heterozygous state. Locally adapted outcrossing maize landraces present valuable diversity reserves. These landraces suffer from strong inbreeding depression when reproduced in small populations or selfed, potentially leading to a reduction in genetic diversity. The production of doubled-haploid (DH) lines presents an even more extreme transition to homozygosity and instantaneously exposes recessive alleles genome-wide. We analyzed the effect of DH production using published data for five European landraces and studied the potential reasons for the loss in diversity. We used genome-wide SNP data of 137 landrace individuals and 404 derived DH lines to show that DH line populations have reduced genome-wide genetic diversity compared to their original landrace population. A likelihood-based grid search test identified 12,585 unique outlier SNPs that drastically changed in allele frequency. Analyzing the fate of the most common haplotype in a region showed an average of 24 % loss and 19 % fixation among them, indicating a reduction of genetic diversity in all populations.

While allelic outliers are similarly abundant in low and high recombination regions, haplotypic outliers show enrichments in specific regions, e.g., a linkage block on chromosome 3 possibly caused by an inversion. We overlapped genomic evolutionary rate profiling (GERP) and phenotypic effect sizes with our outlier statistics but did not find direct evidence of selection against potentially functionally relevant alleles. Our results demonstrate that DH-lines have decreased genetic diversity and cannot capture the full diversity of landraces. While they are not well-suited for the conservation of genetic resources, they are a valuable tool for the introduction of landrace variation into maize breeding programs.

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### Mapping morphological traits characteristic of Mexican highland maize

(submitted by Sergio Pérez-Limón <[checo.spl@gmail.com](mailto:checo.spl@gmail.com)>)

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Maize was domesticated at mid-elevation in western Mexico around 9,000 years ago. Following domestication, maize expanded across the numerous and contrasting environments of Mesoamerica, including colonization of the Highlands (regions above ~2000 m.a.s.l.) of central Mexico. The highland environment presents additional environmental stresses compared to the lowlands, including lower temperature, less precipitation, higher levels of UV radiation, and the risk of frosts. Despite this rather hostile environment, highland maize landraces are capable of growing and being productive.

Mexican highland landraces display characteristic morphological traits - including pronounced stem pigmentation and pubescence and reduced tassel branching - that have been proposed to be part of an adaptive syndrome. In our lab, we are interested in studying the genetic basis of these traits. We have performed Quantitative Trait Loci mapping in a BC1S5 population generated from a cross between B73 and the Mexican highland landrace Palomero Toluqueño. We detected large effect QTL on chromosome 2 for stem pigmentation, QTL for pubescence on chromosomes 7 and 9, and a QTL for tassel branch number on chromosome 7. We speculate on the genes that may underlie these QTL, and present our progress towards fine-scale mapping. We discuss the implications of our findings in the context of the appearance of phenotypic novelty during the development of the Mexican highland maize group.

Funding acknowledgement: Consejo Nacional de Ciencia y Tecnología

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### Mapping of maternal QTLs affecting in vivo haploid induction in maize (*Zea mays* L.)

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Large-scale haploid production was guaranteed by effective haploid induction, thereafter doubled haploid technology has been widely used in maize breeding. Studies have been performed in haploid induction, after dozens of years' endeavor, the inducer gene was cloned and could increase about 10% haploid induction rate (HIR). However, haploid induction rate not only controlled by pollen inducer, but also affected with mother material. In this study, we tried to explain the haploid induction contribution from maternal aspect, which we called maternal haploid inducibility (MHI). According to the screening of maternal haploid inducibility among 20 inbred lines, significant difference between low and high haploid inducibility lines was about 8%. Two extreme materials with high and low maternal haploid inducibility were chosen to cross and then induced to generate F1 haploids. After chromosome doubling we got 135 doubled haploid (DH) lines. These DH lines were planted across four environments and induced by high oil inducer CHO13. After harvest, haploids in each ear of the DH line were identified carefully and haploid induction rate were calculated. The results indicated that the haploid inducibility rate of DH population existed abundant genetic variation region from 10.16 to 19.04% and the heritability of maternal haploid inducibility was approximately 50%. Combined with linkage genetic map constructed by 6K SNP, we performed QTL mapping of maternal haploid inducibility. We detected 15 QTLs in total, two major QTLs from the high parent explained up to 22.14% and 17.44% phenotype variation respectively and only four minor QTLs from the low parent. These results proved to be candidate for fine mapping and the discovery of maternal haploid inducibility QTLs may provide some help for the study of parents interactions in haploid induction.

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### **Mapping the genetic architecture of maize adaptation to the Mexican highlands** (submitted by Sarah Pedersen <[smpeders@iastate.edu](mailto:smpeders@iastate.edu)>)

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After initial maize domestication in the lowlands of the Balsas River Valley, maize expansion led to the encounter of new environments. One of these environments was the highlands of the Mexican Central Plateau. The success of maize in this highland region is thought to be aided by adaptive traits such as reduced flowering time, dark pigmentation, and macrohairs. Shorter flowering time is thought to be adaptive to highland regions due to the shorter growing season plants experience, while increased macrohair density is likely beneficial for retaining moisture around the plant. Increased pigmentation on the leaf sheath could be adaptive for cool climates by increasing the plant's absorbance of radiant energy. To better understand the adaptive role these traits play in highland adaptation, and to understand the genetic architecture of maize adaptation to the highlands of Mexico, we created a mapping population utilizing Palomero Toluqueño, a highland Mexican landrace, and W22. This population is made up of 284 unique BC3S6 lines, allowing for extensive coverage of the gene space. The phenotyping of these lines was done in three locations, including Ames, Iowa, Boone, Iowa, and Metepec, Mexico in order to determine genetic versus environmental effects on the observed phenotypes. DART-seq technology was utilized to generate genotypes and the subsequent datasets were analyzed with R/qtl to determine candidate regions for highland adaption unique to the Mexican highlands.

Funding acknowledgement: National Science Foundation (NSF)

P335

### **Molecular and phenotypic characterization of doubled haploids lines derived from different cycles of recurrent selection of the Iowa Stiff Stalk Synthetic (BSSS) maize population**

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Genetic variability is essential in plant breeding programs, but improvements in crops by plant breeding are usually followed by decreased genetic diversity. A narrow genetic base may lead to a yield plateau, increasing vulnerability to pests and making it difficult to meet new market demands. Assessment of the existing genetic diversity in the available germplasm is fundamental for plant breeding programs. Recurrent selection schemes have proven to be an effective way to improve important agronomic traits while generating a small reduction in genetic diversity when compared to other breeding strategies. The Iowa Stiff Stalk Synthetic Maize Population (BSSS) was developed by intermating 16 inbred lines selected for superior stalk quality and grain yield. The base population has undergone 19 cycles of recurrent selection since 1939 and experienced a significant enhancement in important agronomic traits. Doubled Haploid (DH) technology has become a valuable tool for plant breeding programs because it allows the rapid development of homozygous lines thus significantly reducing the length of a breeding cycle. Additionally, alleles present in a heterogeneous population can be fixed in homozygous and homogenous DH lines allowing a better assessment of the genetic variance and unlocking untapped genetic diversity. Understanding the diversity and relationships between inbred lines within a breeding program is essential for germplasm enhancement. In this study, we will investigate how recurrent selection and DH technology can be optimally combined for line development when using a synthetic population as the breeding germplasm. DH lines with superior performance for agronomic and morphological traits could be identified in this study. Those could be employed for breeding and to broaden the genetic base of Stiff Stalk germplasm thus helping breeders in their quest to raise genetic gains and improve crop yields.



P336

## Natural diversity in Slm1, a highly inducible and autoactive maize immune receptor of the NLR class

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The maize Slm1 gene was identified on its basis to suppress the phenotype of les23, a recessive lesion mimic mutation. The cloning and characterization of genes underlying both loci revealed that while Slm1 encodes an immune receptor of the NLR (nucleotide binding leucine rich repeat receptor) family, les23 is defective in a homolog of RIN4, which was initially identified as an R gene-interacting protein in Arabidopsis. Activation of NLRs triggers the induction of a programmed cell death response termed as the hypersensitive response (HR). In-depth analysis of the interaction of Slm1 and les23 reveal that the immune receptor encoded by Slm1 is an autoactive NLR that is normally prevented from triggering HR by LES23. But when les23 is defective, SLM1 NLR cannot be kept in check and HR cell death ensues spontaneously. However, if SLM1 is defective, no HR lesions are produced regardless of whether les23 is functional or not. The relative expression of Slm1 and Les23 is suggestive of the existence of an HR-modulating ‘immunostat’ during pathogen infection. Interestingly, the Slm1 gene is highly polymorphic in the NAM founder set, with more than half of the inbreds containing large indels. The nonfunctional nature of these Slm1 alleles was revealed by screening F2 populations that were derived from crosses of the NAM inbreds with a double slm1les23 mutant. This analysis also showed that the slm1 alleles of many other NAM inbreds had a weak or delayed autoimmune phenotype. One interpretation of these results is that the fitness cost of the WT Slm1 allele is too high to be retained in elite breeding lines.

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

P337 

## Navigating the maize of short-season ancestry

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Preliminary analysis on maturity stratified maize lines suggest that the early germplasm pool has broader ancestry than what was originally proposed. As part of the Genomes to Fields (G2F) project a series of NC Design II yield trials were conducted using lines from recently expired PVP certificates or their second generation lines, chosen based on diversity of originator and on usage in commercial hybrids. The lines were also stratified to generate a set of hybrids for testing in the Early G2F environments, the Intermediate and the Late G2F testing environments. Due to selection for earliness and a genetic bottleneck in the early lines, it is accepted that the ancestry of this group is narrow and consists primarily of ancestral early lines. However, the DII Early set exhibited the greatest genetic variation as a percentage of the total variation for grain yield, plant height, and days to silking and was the only set to have exhibited significant additive genetic variation in both the males and females for grain yield. This has led to the hypothesis, the modern early season germplasm base has founder lines that are not part of the founders of the corn belt dent germplasm base. A sample of short season germplasm inbred lines from AAFC, NDSU, early flowering off- PVP lines, and CG lines derived from short season commercial hybrids will be used to determine the relationship of the this germplasm pool with the proposed 7 founder lines and what its most likely founder lines are.

Funding acknowledgement: NSERC, OMAFRA



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## Optimal designs for genomic selection in hybrid crops

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Plant breeding is enhanced by integrating different scientific innovations and enabling tools. A critical question emerged with the increased capacity in genomics and biotechnology: how to efficiently establish genotype-to-phenotype relationship so that prediction can be made to guide our exploration of the enormous genotype space. Here we show that representative subset selection can be applied to training set design to enhance performance prediction of hybrids. Specifically, we designed three methods of representative subset selection: Maximization of connectedness and diversity (MaxCD) from the genetic mating scheme perspective; Partitioning around medoids (PAM) from cluster analysis; and Fast and unique representative subset selection (FURS) from graphic network analysis. We phenotyped a set of 276 maize hybrids generated by crossing founder inbreds of Nested Association Mapping (NAM) populations for flowering time, ear height, and grain yield. With 10,296,310 SNPs available for the parental inbreds, we explored the patterns of genomic relationships and phenotypic variation to establish training samples based on three representative subset selection methods. Our analysis showed that training set designs outperformed random sampling and earlier methods that either minimize the mean of prediction error variance or maximize the mean of generalized coefficient of determination. Similarly, analyses with 2,556 wheat hybrids from an early-stage hybrid breeding system and 1,439 rice hybrids from an established hybrid breeding system validated the advantages of the new methods. With representative subset selection, effective genomic prediction models can be established with a training set 2%~13% of the size of the whole set. Enhanced by design thinking, genomic selection may reshape the plant breeding pipeline by enabling the efficient exploration of the enormous inference space of hybrid combinations. Data mining and design optimization can offer additional guidelines for plant biology research.

Funding acknowledgement: National Science Foundation (NSF)

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## Parental RNA polymerase IV conditions heterotic traits

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Maize RNA polymerase IV (RNAP IV) affects genome-wide RNAP II-based transcription<sup>1</sup> and defines expression patterns of specific alleles<sup>2,3</sup>. RNAP IV also mediates and maintains transcriptional repression of alleles undergoing paramutation<sup>4</sup> – a meiotically-heritable change in gene regulation facilitated by *trans*-homolog interactions<sup>5</sup>. Paramutation behaviors of certain *red1* and *purple plant1* alleles controlling anthocyanin pigment production provide clear examples of single locus heterosis<sup>6,7</sup>, fueling speculations that similar behaviors contribute to hybrid vigor<sup>7,8</sup>. Here I report field trial results showing that absence of RNAP IV function in B73 parents mitigates heterotic traits of B73/Mo17 hybrids. Hybrid gains in plant height were contributed by RNAP IV function in either parental sporophyte whereas preliminary results indicate that only maternal RNAP IV affects non-hybrid offspring. Approximately 10% of hybrid grain yields were dependent on maternal RNAP IV. Understanding the genomic features that recruit RNAP IV and the various roles both RNAP IV and RNAP IV-generated 24 nucleotide RNAs play in defining RNAP II transcription patterns promises novel opportunities for predicting and controlling biomass production. Indeed, Shull noted that “the differences between uniting gametes responsible for heterosis need not be Mendelian in nature”<sup>7,9</sup>.

1. Erhard *et al.* 2015 *Genetics* | 2. Parkinson *et al.* 2007 *Dev Biol* | 3. Erhard *et al.* 2009 *Science* | 4. Erhard *et al.* 2013 *Plant Cell* | 5. Hollick 2017 *Nat Rev Genet* | 6. Styles and Brink 1969 *Genetics* | 7. Hollick and Chandler 1998 *Genetics* | 8. Kermicle and Alleman 1990 *Development* | 9. Shull 1948 *Genetics*

Funding acknowledgement: National Science Foundation (NSF), Ohio State Foundation

P340 

## Phenomics of stomata and water use efficiency in C4 species

(submitted by Andrew Leakey <[leakey@illinois.edu](mailto:leakey@illinois.edu)>)

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Water use efficiency (WUE), which is physiologically distinct from drought tolerance, is a key target for improving crop productivity, resilience and sustainability. This is because water availability is the primary limitation to crop yield globally and irrigation uses the largest fraction of our limited freshwater supply. The exchange of water and CO<sub>2</sub> between a leaf and the atmosphere is regulated by the aperture and pattern of stomata. Mechanistic modeling indicates that stomatal conductance could be reduced or stomatal movements accelerated to improve water use efficiency in important C<sub>4</sub> crops such sorghum and sugarcane. While molecular genetics has revealed much about the genes regulating stomatal patterning and kinetics in Arabidopsis, knowledge of the genetic and physiological control of WUE by stomatal traits in C<sub>4</sub> crops is still poor. Understanding of natural diversity in stomatal traits is limited by the lack of high-throughput phenotyping methods. Two novel phenotyping platforms were developed. First, a rapid method to assess stomatal patterning in three model C<sub>4</sub> species grown in the field – maize, sorghum and setaria has been implemented. The leaf surface is scanned in less than two minutes with an optical tomographer, generating a quantitative measurement of a patch of the leaf surface. An algorithm was designed to automatically detect stomata in 10,000s of these images via training of a neural network approach. Second, a thermal imaging strategy, to rapidly screen the kinetics of stomatal closure in response to light has been developed. We identified genotype to phenotype associations for stomatal patterning, leaf gas exchange and canopy water use through quantitative trait loci and genome wide association studies. Transgenically modified expression of stomatal patterning genes has produced sorghum with greater WUE. These plants were grown in a new field facility for comprehensive evaluation of leaf, root and canopy WUE traits under Midwest growing conditions in summer of 2018.

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

P341

## Phenotypic diversity of pakistani maize (*Zea mays* L.) breeding lines for heat stress and agronomic traits

(submitted by Zubair Ahmed <[zubairnarc15@gmail.com](mailto:zubairnarc15@gmail.com)>)

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Maize has significant importance for countries like Pakistan, where rapidly increasing population, poultry and livestock have already outstripped the available food and feed supplies. This crop is affected by various abiotic and biotic stresses, but the major yield-reducing abiotic factor is heat stress. Global warming and climate change are well-known phenomena, and South Asia is among the most affected regions of the world. Spring maize in Pakistan is exposed to extreme heat stress at the flowering stage during the months of May and June. In the hot air during pollination, the delicate pollen grains desiccate immediately after they are released from the maize tassel. High temperature can also affect other stages of plant growth and development. Increasing daily and monthly temperature threaten to cause a severe reduction in crop productivity in the near future, which is a big threat to food security in this region. This study is conducted to assess which traits are key for mitigating heat stress. More than 380 maize accessions of various maturity classes, QPM lines, hybrid parental lines, drought tolerant, low nitrogen, borer resistant, open-pollinated varieties and a large number of local germplasm was collected from MSM&F program NARC, CIMMYT Pakistan and Plant Genetic Resources Institute (PGRI), NARC, Pakistan. These accessions were planted at two sowing dates (normal and late), at two locations (NARC, Islamabad and University of Agriculture, Peshawar) during spring 2017 and 2018. Various phenological, morphological, physiological, yield and stress-related traits were recorded and analyzed. Future work will include genotyping and mapping genetic control of heat-related traits and genomic prediction. This work will also help in the identification and development of heat tolerant OPVs/inbred lines which will ultimately be useful for high yielding OPV/hybrid development for local conditions.

P342 

## Population divergence and inbreeding depression in maize heterosis

(submitted by Fernando Silva Aguilar <[fsilvaag@iastate.edu](mailto:fsilvaag@iastate.edu)>)

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Heterosis, first described by Shull in 1909, refers to the superiority of the hybrid over parents. Heterosis, depends on the level of dominance and on differences in allele frequencies between parents. The objective of this experiment was to estimate the contribution of baseline and panmictic heterosis to heterosis for yield and to estimate the reduction in grain yield when selfing or random mating the F1 generation. Heterosis was estimated as panmictic-midparent heterosis (PMPH) and inbred-midparent heterosis (IMPH). PMPH was estimated as the difference between the F1 and the two random mated parental populations, while for the IMPH the midparent value was estimated after selfing the populations until homozygosity. Estimates of baseline heterosis were obtained as the difference between IMPH and PMPH. Estimates of PMPH were  $2.4 \pm 0.27$  and  $4.87 \pm 0.25$  Mg/ha for the population-by-population and population-by-inbred crosses, respectively, suggesting that all the F1 generation express a superiority in grain yield compared to the mean of its parents. Equally, due to a significant reduction in the midparent mean when the parental populations were selfed to homozygosity, the estimates of IMPH increases to  $5.15 \pm 0.45$  and  $6.49 \pm 0.32$  Mg/ha for the population-by-population and population-by-inbred crosses, respectively. Genetic expectations of PMPH reflects only the importance of genetic divergence ( $4d\Delta^2$ ) while IMPH is a function of divergence and inbreeding depression ( $2pqd$ ). The increase in the IMPH suggests that inbreeding depression in the parental populations was stronger than genetic divergence. Baseline heterosis, which is the inbreeding depression minus divergence, range from  $2.76 \pm 0.51$  to  $1.62 \pm 0.28$  Mg/ha for population-by-population and population-by-inbred crosses, respectively. Finally, the values for baseline heterosis suggests that the recovery from inbreeding depression was more important than the genetic divergence for the population-by-population cross, while for population-by-inbred crosses it was the opposite.

Funding acknowledgement: United States Department of Agriculture (USDA)

P343 

## Predictive ability of hybrid performance via combining ability models in interaction with environmental data

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<sup>1</sup> Predictive ability of Hybrid Performance via Combining Ability Models in Interaction with Environmental Data

The prediction of hybrid performance can enhance breeding programs by allowing the selection of the best set of parents for developing the best progeny for a particular trait (yield, pest resistance, drought tolerance, etc.) in target environments. Genomic Selection is an emergent tool that have shown advantages over conventional breeding techniques based on phenotype for increasing genetic gains of developed population. Potentially, this tool also can be used in the selection process for developing hybrid populations by screening candidate hybrids based on the genetic profiles of their parents without having to develop and plant these materials in fields. Conventional GS approach was developed assuming that calibration and testing sets are observed under same environmental stimuli; however, this assumption is not feasible in most of the cases. In general, the environmental conditions change from one year/site to other causing changes in the ranking of the performance of hybrids. The presence of significant genotype-by-environment interaction (GxE) complicates the labor of the breeders for selecting the parents for developing good hybrids. In this research, I propose a series of models that take advantage of the (i) general and specific combining abilities of the parents (inbred) (ii) in presence of significant GxE interaction via co-variance structures. Data from the genomes to field G2F project, which also includes weather information hourly recorded, was used for testing our proposed models. Four realistic scenarios that breeders face in fields were considered: CV2 prediction of incomplete field trials [tested hybrids in tested environments]; CV1 prediction of newly developed hybrids in tested environments; CV0 prediction of tested hybrids in new environments; CV00 prediction of new hybrids in new environments. The relative improvements of the proposed models compared with the traditional hybrid model in terms of predictive ability were: 75% [CV2], 95% [CV1], 57% [CV0] and 207% [CV00].

Funding acknowledgement: Iowa Corn Growers Association

P344 

## **Production of maize chromosome segment substitution line populations for the identification of loci associated with multiple disease resistance**

(submitted by Peter Balint-Kurti <[pjbalint@ncsu.edu](mailto:pjbalint@ncsu.edu)>)

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Southern Leaf Blight (SLB), Northern Leaf Blight (NLB), and Gray Leaf Spot (GLS) are among the most important diseases of corn worldwide. Previously highly significant genetic correlations between resistance levels to each of these diseases were identified in a panel of 253 diverse maize inbred lines. In order to identify loci underlying disease resistance in some of the most multiple disease resistant (MDR) lines we created a set of chromosome segment substitution line (CSSL) populations in multiple disease susceptible (MDS) backgrounds. Four MDR lines (NC304, NC344, Ki3, NC262) were used as donor parents and two MDS lines (Oh7B, H100) were used as recurrent parents to produce eight BC3F4:5 CSSL populations comprising 1,611 lines in total. These populations are all available from the genetic stock center. Each population was genotyped and assessed for each disease in replicated trials in two environments. Multiple quantitative trait loci (QTL) for disease resistance were detected for each disease in most of the populations. Seventeen QTLs were associated with variation in resistance to more than one disease (SLB/NLB: 2; SLB/GLS: 7; NLB/GLS: 2 and 6 to all three diseases). Significant correlations were observed between disease scores and also between marker effects for each disease. The number of lines that were resistant to more than one disease was significantly higher than expected by chance. F2:3 populations were subsequently produced and used to validate and better measure the parameters of several of these QTL.

Together, these findings reinforce our previous conclusions that loci associated with resistance to different diseases are clustered in the genome more often than would be expected by chance. Nevertheless true MDR loci which have significant effects on more than one disease are still much rarer than loci with single disease effects.

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

P345 

## **Purifying selection and its phenotypic consequences on micronutrients in maize**

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Maize is one of the most important crops all around the world. The incremental demands for the human diet, animal feed, and industrial feedstock require the yield and quality of maize to be continuously enhanced. During the processes of maize domestication and improvement, deleterious alleles, likely affecting fitness and yield, have been purged due to purifying selection. The effects of the purifying selection on quality traits such as the compositions of micronutrients, however, have been less explored, especially under different nitrogen conditions. Public data indicate that some of the compositions of micronutrients in kernels are declining during the past several decades. Based on this observation, we hypothesized that beneficial alleles for micronutrients might have been selected against unintentionally if they are in linkage disequilibrium with the deleterious alleles. To address this hypothesis, we conducted large-scale field experiments using the maize diversity panel under two nitrogen conditions (N+ and N-) with two replications. We collected micronutrients and other phenotypic traits using high-throughput phenotyping approaches. Then, we performed genome-wide complex trait Bayesian analysis to estimate parameters of genetic architectures using genome-wide SNPs including putative deleterious variants. Results from this study will contribute to understanding the purifying selection for deleterious and their effects on micronutrients.

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

P346

## **QTG-seq accelerates QTL fine mapping through QTL partitioning and whole-genome sequencing on bulked segregant samples**

(submitted by Xi Wang <[13297032291@163.com](mailto:13297032291@163.com)>)

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Deciphering the genetic mechanisms underlying agronomic traits is of great importance for crop improvement. Most of these traits are controlled by multiple quantitative trait loci (QTL), and identifying the underlying genes by conventional QTL fine-mapping is time-consuming and labor-intensive. Here, we devised a new method we named quantitative trait gene sequencing (QTG-seq) to accelerate QTL fine mapping. QTG-seq combines QTL partitioning to convert a quantitative trait into a near-qualitative trait, bulked segregant sequencing on a large segregating population, and a robust new algorithm for identifying candidate genes. Using QTG-seq, we fine-mapped a plant height QTL in maize (*Zea mays* L.), qPH7, to a 300-kb genomic interval and verified that a gene in that region encoding an NF-YC transcription factor was the functional gene. Molecular evidence suggested that qPH7 might influence plant height by interacting with proteins encoded by a CO-like gene and an AP2 domain containing gene. Selection analysis indicated that qPH7 was subject to strong selection during maize improvement. In summary, QTG-seq provides an efficient method for QTL fine-mapping in the era of “big data”.

Key words: Quantitative trait gene (QTG), Quantitative trait locus (QTL), QTL fine-mapping, QTL partitioning, Whole genome sequencing

Funding acknowledgement: National Natural Science Foundation of China



P347

### **QTL analysis of maize seedling cold tolerance using Vigor: a machine vision assay for seedling emergence.**

(submitted by Jeffery Gustin <[jgustin@ufl.edu](mailto:jgustin@ufl.edu)>)

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Seedling emergence is a critical stage in the establishment of a successful crop. Germination and robust seedling establishment are selected traits during the development of new varieties but with inefficient, largely manual methods. We developed an in-lab, soil-based machine vision platform to automatically measure emergence parameters, including rate, average time, duration, and percent. The assay is scalable, accommodates chemical or environmental treatments, and can be used with different soil types. Individual cameras monitor 168 kernels for time-lapse imaging, and we have scaled to twelve cameras to monitor 2,016 kernels in parallel. Time-lapse images are processed by a custom, public application in the CyVerse cyber infrastructure. Maize seedling percent emergence is measured with a 2% False Negative Rate and mean emergence time is measured to within 3.5 hours of human scores. We used the assay to evaluate cold tolerance in the Intermated B73xMo17 Recombinant Inbred (IBM RIL) population consisting of 276 RILs. Kernels were sowed into 5C soil for 5 days followed by transferred to 24C for emergence. This harsh, short term treatment induced substantial variation in emergence percentage among RILs and less variation in emergence time. Quantitative Trait Loci (QTL) that control emergence percentage under cold conditions were detected that overlap with previously identified QTL. In addition, our treatment conditions revealed an apparently novel QTL on chromosome 10 that had a large impact on multiple emergence traits in both warm and cold conditions. Novel QTL, such as this, may be the result of conducting the experiment in controlled soil environment rather than semi-sterile lab conditions or heterogeneous field conditions

Funding acknowledgement: National Science Foundation (NSF)

P348 

### **Repeat depletion for high-throughput sequencing of exonic and low copy regions of the maize genome**

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Resequencing of the maize genome is increasingly becoming an operational method for genotyping and genomic analysis. This is largely a result of reduction in sequencing costs and development of analytical tools to leverage this data. For example, as more samples are sequenced, haplotype databases are improved allowing for accurate imputation of low-depth sequencing data (skim sequencing) to genome-wide markers. The genome of maize, however, is notoriously repetitive and up to 80% of the short-reads generated from a resequencing project may be expected to map to high-copy regions and be discarded during bioinformatic analysis. Furthermore, genic and gene-proximal variants are more likely to affect important traits and can be used during imputation analysis. To improve resequencing and skimming in maize, we developed a method to deplete repeat-like regions from the genome during library construction for next-generation sequencing. The library preparation process itself is fast, automatable and does not require mechanical shearing of the DNA. To validate this method, we built repeat-depleted libraries for three replicates of the B73 genome and compared the results with standard libraries without repeat depletion. For all libraries, 60 million paired-end 2x150 Illumina HiSeq X reads were generated. For the amount of data generated, the average sequencing depth for fully sequenced exons went up from 15X (sd=0.5) to 65X (sd=2) when comparing depleted to non-depleted data, suggesting that repeat-depleted libraries are ~500% more efficient in sequencing exons. In the repeat-depleted samples, 90% of the exons were fully sequenced, highlighting the specificity of the reaction in depleting repetitive DNA. Additionally, of the 5.3 million HapMap SNPs in exonic regions, we retained 95.5% in the treated libraries. Coupled with our streamlined library preparation, the depletion protocol is an effective approach to conduct maize skim sequencing and re-sequencing, concentrating the sequencing efforts on the most important regions of the genome.

P349

### **Rootless1 as a breeding target for reduced crown root number in maize**

(submitted by Adam Bray <[abray@danforthcenter.org](mailto:abray@danforthcenter.org)>)


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New traits are needed to speed development of climate resilient plant varieties. Roots remain an untapped resource for plant improvement. In particular, reduced crown root number has been proposed as a target for breeding plants with deeper and cheaper roots. The classical maize mutant *rootless1* (*rtl*) was reported by Jenkins in 1930 to have severely reduced crown and brace root numbers on the stem. We have confirmed the gene through complementation experiments with *rtl* and Ac/Ds tagged lines. Additionally, quantitative trait loci (QTL) for crown root number have been identified that contain functional copies of the gene. Together our X-ray Micro CT and qRT-PCR data suggest that *rtl* acts in early crown root primordium, preventing elongation. Additionally, we are testing near-isogenic lines to confirm that *Rtl* is involved in regulating crown root number under the identified QTL. The available body of evidence suggests that breeders could target *Rtl* to reduce crown root number and generate maize lines with improved water capture from deeper soil layers.

Funding acknowledgement: National Science Foundation (NSF)

P350 

### **Shared genetic control of root system architecture between *Zea mays* and *Sorghum bicolor***

(submitted by Zihao Zheng <[zhzheng@iastate.edu](mailto:zhzheng@iastate.edu)>)

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Determining the genetic control of RSA (root system architecture) in plants via large-scale GWAS (genome-wide association study) requires high-throughput pipelines for root phenotyping. We developed CREAMD (Core Root Excavation using Compressed-air), a high-throughput pipeline for the cleaning of field-grown roots and COFE (Core Root Feature Extraction), a semi-automated pipeline for the extraction of RSA traits from images. CREAMD COFE was applied to diversity panels of two crops; the maize and sorghum diversity panels consisted of 369 and 294 genotypes, respectively. Six RSA-traits were extracted from images collected of >3,300 maize roots and >1,470 sorghum roots. SNP-based GWAS identified 87 TAS (trait-associated SNPs) in maize, representing 77 genes and 115 TAS in sorghum. An additional 59 RSA-associated maize genes were identified via eRD-GWAS. Among the 136 maize RSA-associated genes (or their homologs), 20 (15%) are known to affect RSA in maize or other species. In addition, 13 RSA-associated genes are co-regulated with genes previously shown to affect RSA and 10% of RSA-associated genes are themselves trans-eQTL for another RSA-associated gene. Finally, the finding that RSA-associated genes from maize and sorghum included seven pairs of syntenic genes, demonstrates the conservation of regulation of morphology across taxa.

Funding acknowledgement: ARPAAE

P351 

## **Spatial statistical analysis in the evaluation of tropical maize hybrids for agronomic and N-related traits under low and high N input**

(submitted by Leandro Zuffo <[zuffo@iastate.edu](mailto:zuffo@iastate.edu)>)

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Maize is an important crop which is cultivated around all world. It is the cereal with highest world production (1.04 Mg). Nitrogen (N) is the nutrient mostly demanded by maize and the main cause of grain yield limitation. Field gradients are common at experimental areas, especially under stressful conditions. Spatial analysis has been applied in several studies to correct residual errors and to decrease the bias estimates. Our objective was evaluated the spatial analyses to correct the spatial auto-correlation of tropical maize hybrids under low and high N input. We evaluated 112 tropical maize hybrids provided from 18 seed companies for agronomic and N-related traits at the experimental area of Federal University of Vicosa in two growing seasons (2015/2016 and 2016/2017). At both growing seasons the hybrids were evaluated at two N levels: low N (LN) and high N (HN) (20 kg ha<sup>-1</sup> and 200 kg ha<sup>-1</sup> of N, respectively). The experimental design was an alpha lattice with three replicates. Plot size was 6.4m<sup>2</sup> (two rows spacing of 0.8 m and length of 4 m). We tested three different models: I) randomized complete block, II) first-order autoregressive model in two dimensions (AR1xAR1) and III) AR1xAR1 with nugget effect. Comparing the models by Akaike Information Criterium (AIC), model II and III were superior to the model I for all traits and, the model III was superior or equal than model II for almost all traits. When the AICs between model II and III was equals the model III showed lower residual variance, even when the model II was superior than model III the residual variance was the same. We concluded that spatial analysis is a powerful tool to correct the spatial auto-correlation at field experiments for agronomic and N-related traits under low and high N input.

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P352

## **Structural variation analysis in the Wisconsin Diversity Panel using short read sequence data and multiple *de novo* genome assemblies**

(submitted by Christine O'Connor <[coconnorc@umn.edu](mailto:coconnorc@umn.edu)>) (Presenter: [Christine O.](#)

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Structural and copy number variation is pervasive throughout the maize pangenome and may be important for phenotypic diversity. With the publication of more and more maize reference genomes, it has become increasingly clear that a single reference genome does not capture all the variation across maize, a pattern seen in many other crop species. Understanding the depth and breadth of structural variation across the maize pangenome can help us identify the genetic basis of important phenotypic variation. In this study, we are creating a comprehensive catalogue of small structural variants in maize in a set of diverse inbred lines. We are making full use of the genomic resources available in maize – we have whole genome sequencing data from over 100 maize genotypes and each genotype has been aligned to four maize reference genomes. Our results show that there can be a one and a half-fold difference in the number of structural variants identified for the same genotype aligned to two different reference genomes, highlighting the importance of incorporating more than one reference genome when identifying structural variation in a maize genotype. Beyond gaining an understanding of the breadth of variation in number and location of structural variants across the maize pangenome, the structural variant panels created for each maize genotype will also be used in future work to connect structural variation to phenotypic variation.

Funding acknowledgement: National Science Foundation (NSF)

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### The genetic architecture of nodal root number in maize

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The maize nodal root system plays a crucial role in the development of the above ground plant and determines the yield via the uptake of water and nutrients in the field. However, the genetic architecture of the maize nodal root system is not well understood, and it has become the ‘dark matter’ of maize genetics. Here, a large teosinte-maize population was analyzed, and high-resolution mapping revealed that 62 out of 133 quantitative trait loci (QTLs), accounting for approximately half of the total genetic variation in nodal root number, were derived from QTLs for flowering time, which was further validated through a transgenic analysis and a genome-wide association study. However, only 16% of the total genetic variation in nodal root number was derived from QTLs for plant height. These results gave a hint that flowering time played a key role in shaping nodal root number via indirect selection during maize domestication. Our results also supported that more aerial nodal roots and fewer crown roots might be favored in temperate maize, and this root architecture might efficiently improve root-logging resistance and the ability to take up deep water and nitrogen under dense planting.

Funding acknowledgement: The National Key Research and Development Program of China

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### Towards large scale corn root phenotyping: Designing and implementing genomic prediction

(submitted by Andrew Herr <[awherr@iastate.edu](mailto:awherr@iastate.edu)>)

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Root structure and root traits have a significant impact on the productivity of corn in a given environment. Roots affect how a plant will react to drought, flooding, and heat stress episodes, and are responsible for nutrient uptake. A robust, effective root system creates the potential for high yield. Two essential traits to test for in a root system are root angle and root biomass. Root biomass is an indicator of the size of a plant's root system and the access it will have to nutrients. Root angle provides structural support and profoundly impacts water absorption through changing the depth at which the roots will extend. In the present plant breeding industry, phenotypic traits can be selected for and improved through genomic selection (GS). In the last decade, GS has become a cost-effective tool available to plant breeders. The objective of this study is to determine if GS can effectively be used to make predictions with below ground traits like root angle and root biomass in a selected subset of hybrids. To achieve this goal, 30 expired Plant Variety Protection maize lines representing Iodent and Stiff Stalk heterotic groups were mated in a full diallel. Novel algorithms were applied to select a subset of the F1s to serve as a training data set to perform GS on the remaining F1s. In both the 2017 and 2018 growing season, we harvested 528 root systems. Roots were collected at maturity and phenotyped using a high-throughput, custom imaging analysis script. This collected phenotypic data will be combined with molecular marker data to perform GS. The proposed research will be vital to a plant breeder's ability to assess and improve corn root productivity while using fewer resources.

Funding acknowledgement: Department of Energy (DOE), ISU Raymond F. Baker Center for Plant Breeding, DuPont Pioneer, ISU Plant Sciences Institute

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## Towards predictive maize breeding in the GxExM space

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Genomic selection models have revolutionized plant improvement, giving breeders a window into a genotype's performance prior to phenotyping, and sometimes even before planting. Similarly, whole crop physiological models allow farmers and agronomists to evaluate *a priori* the risks and benefits of planting a certain species in a given year, and the implementation of new management practices. Genomic selection performs well in the context of a single environment with many genotypes, while physiological models have high accuracy for a few well studied genotypes across many environments. Attempts to integrate genomic selection and physiological models for better prediction and understanding of Genotype by Environment by Management interactions (GxExM) have been hampered by deeply rooted methodological differences between the two modeling frameworks, and the availability of genotype, phenotype, soil, weather, and management data at the scales necessary to meet the requirements of both types of models. Several recent projects, in particular the maize Genomes to Fields initiative (G2F, [genomes2fields.org](http://genomes2fields.org)), have attempted to gather data at scales similar to what is needed for such models. Here, we evaluate the data gathered in some of these projects for suitability in a combined genomic selection and whole crop physiological modeling framework. We also present the ongoing development of computational tools necessary for the implementation of this modeling framework, and preliminary results from its use.

Funding acknowledgement: National Science Foundation (NSF)

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## Understanding and predicting phenotypic plasticity with Joint Genomic Regression Analysis (JGRA)

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Plants sense and integrate environmental cues, and alter their development accordingly. Genes that are influenced by the environment exhibit variation in their response to external cues, which is the basis of genotype-by-environment interaction (GxE). The objective is to identify environmental factors to use in a quantitative index that has biological meaning and is relevant to the field setting. Such an index would permit trait prediction in new environments. Our research used the large, publically available maize dataset Genomes 2 Fields (G2F). The G2F project started in 2014 with 13,000 plots evaluated in 19 locations and by 2017 has grown to over 21,000 plots evaluated in 38 locations. A weather station in each location collects 10 environmental factors every 30 minutes and inbred parents have been genotype via genotype-by-sequencing. The combination of multi-environment phenotyping, envirotyping and genotyping makes the G2F dataset ideal to search for a continuous environmental index and assay the effect of such an index on predicting phenotypic plasticity. Using 884 hybrids evaluated in 2014 and 2015 across 33 environments, we found that temperature (GDD) between 6 and 35 days after planting (DAP) explained 85% of variation for flowering time. Using GDD between 6 and 35 DAP as an environmental index, we achieved an overall prediction accuracy of 0.83 for untested genotypes in untested environments via k-fold validation. We obtained similar prediction accuracies when the G2F dataset was subset by tester. We call the incorporation of an environmental index with genomic regression, Joint Genomic Regression Analysis (JGRA). This is the first application of JGRA to hybrid genotypes and future objectives are to apply the JGRA to traits beyond flowering time.

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

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## **Understanding compositional changes during the alkaline cooking of maize (*Zea mays* L.) to mitigate acrylamide formation**

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Acrylamide has been found to increase the risk of several cancers in lab animals and was first discovered in food products in 2002. This compound is a by-product formed during the Maillard non-enzymatic browning reaction when reducing sugars and free amino acids react at high temperatures. Of the available free amino acids, asparagine has been proposed as a key substrate in the acrylamide formation pathway. Traditionally, acrylamide levels in maize based food products have been managed with the use of the enzyme asparaginase. However, the high cost of asparaginase coupled with growing consumer demand for processed foods with natural ingredients has necessitated the need for alternative acrylamide reduction strategies. Little is known about natural variation that exists in maize for these precursors or how the precursors are modified throughout the cooking process. To characterize variation in substrate content in raw kernels as well as understand how levels of reducing sugars and free amino acids change throughout nixtamalization, 120 inbred lines with extreme values based on NIR equations for total sugars, starch, and nitrogen content were selected. These samples were then processed through a small-scale bench top cooking protocol with subsamples taken at key steps during cooking. Quantitative measurements of reducing sugars, free amino acids, total starch, and protein content are being measured on each subsample with various analytical procedures. Preliminary results have shown in raw kernels asparagine, glucose, and fructose ranged from 0.13 to 1.08, 0.58 to 5.56, 0.16 to 3.04 g/100g flour respectively. Variation across genotypes has been observed for compositional changes during cooking, with an average 6-fold decrease in asparagine and 19-fold decrease in reducing sugars. Elucidating how levels of these substrates change throughout the cooking process and the relationship with raw kernel compositional traits will provide valuable information for future breeding efforts of food grade maize.

Funding acknowledgement: PepsiCo Inc.

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## **Using spontaneous haploid genome doubling to access favorable alleles in exotic germplasm**

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Exotic maize (*Zea mays* L.) germplasm, usually open-pollinated varieties (OPV), represent a massive pool of largely untapped genetic diversity. The high genetic load and heterozygosity of OPV individuals hampers their use in breeding process. Doubled haploids (DH) have been shown to be an effective tool for maize line development and alternative for exotic germplasm exploitation, where rapid homozygous fixation could capture most the variation present in the original source. A major bottleneck on DH process is the restoration of male/female fertility through artificial genome doubling (AGD). Spontaneous haploid genome doubling (SHGD) would eliminate the need of artificial chromosome doubling, allowing direct seeding of haploids, simplifying the production steps, reducing time and costs. The main objective of this study is to quantify the effect SHGD on germplasm exploitation and on selection of superior maize inbred lines. Testcross performance evaluation was conducted for estimate the genetic variance among progenies derived from two breeding methods (DH and SSD) and the two chromosomal doubling methods (artificial and spontaneous). Results will be compared with modern hybrids to determine whether lines with SHGD genes have the same breeding potential as lines without those genes. Genotype-by-sequencing data will be used for the investigation of a possible bottleneck associated with the haploidization process, and identification of possible segregation distortion related with SHGD genes.

Funding acknowledgement: United States Department of Agriculture (USDA)



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## Whole genome sequencing and *de novo* assembly for *Andropogoneae* species

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The *Andropogoneae* clade includes maize and sorghum and captures ~18 million years of evolutionary history, giving us more genome sequence diversity information than inner species populations. We are currently in the process of performing whole genome sequencing on hundreds of the *Andropogoneae* species. We will report Oxford Nanopore MinION sequencing and assemblies for several of these. The software wtdbg2, Canu, and miniasm have been used and compared for *de novo* assembly. Aiming to uncover the genetic regions and *cis*-regulation elements, we mapped the coding sequence (CDS) of a set of conserved maize genes to the assemblies using the software minimap2 and the minimum distances between the gene and the assembled contig edges were measured. The number of genes being mapped and the minimum distance are being used as indications of assembly quality. For cost efficiency, we are trying to determine the impact of read length and read coverage on assembly. When compared with coverage, the assembly N50 and minimum distance is more sensitive to read length. We will apply sensitive sequence alignment methods to align the potential regulatory regions of homologous genes. With conserved regulation elements, we could learn how each individual gene is being expressed and regulated, and work toward predicting and ranking deleterious and adaptive variants.

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

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## X-ray imaging across scales in maize and sorghum biology: centimeters to micrometers

(submitted by Keith Duncan <[kduncan@danforthcenter.org](mailto:kduncan@danforthcenter.org)>)

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The accurate and precise analysis of complex plant structures is difficult if researchers are restricted to the measurement of 2D features, regardless of the resolution of the imaging technology being used. Detailed 3D volumes of plant samples using X-ray imaging are now achievable that span the range of feature resolution from tens of centimeters down to single micrometers. We are using X-ray tomography (XRT), X-ray micro computed tomography ( $\mu$ CT), and X-ray microscopy (XRM) to study maize and sorghum development across a wide range of imaging scales. Our large scale XRT/ $\mu$ CT system is used to scan thousands of excavated maize root crowns and sorghum panicles in the 80 $\mu$ m-110 $\mu$ m range. The resulting 3D volume data is being processed in feature extraction pipelines to help identify genetic elements that influence panicle traits and root system architecture. Existing sample preparation techniques from electron microscopy have been adapted for generating high resolution 3D volumes with the XRM, allowing analysis of the nodal plexus of maize brace root vasculature in the 10 $\mu$ m-50 $\mu$ m range. XRM is also being used to image early stages of inflorescence development in sorghum panicles, and the growth and elongation of root tip cells from maize lines that differ in mature root system architecture, both in the 1 $\mu$ m-20 $\mu$ m range. The continuing challenge remains the accurate and high-throughput segmentation of biologically relevant features from 3D X-ray data. Cutting-edge machine learning and computer vision results are presented. We are also developing Virtual Reality (VR) as a comprehensive vehicle for data visualization and analysis, as well as for education and outreach. A demonstration will be provided.

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**P361**

### **Xenia effect on oil content in maize embryo by hetero-fertilization**

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The hetero-fertilization is a phenomenon that the egg and polar nuclei fuse with the different genotypes sperms, respectively, resulting in different genetic backgrounds of developing embryos and endosperm, which pave the way to studying the dosage effect and heterozygous effect on maize embryo. In this study, 4 inbred lines (2 common inbred lines and 2 high oil content inbred lines) were self-pollinated and crossed to study the xenia of oil content, in addition, 1 haploid inducer line and 1 high oil content inbred line were used to generate the haploid and hetero-fertilize kernels for studying the genetic effects of oil content. The result showed that the oil content of crossed kernel was higher than that of selfed kernel, and the over female heterosis of oil content had a positive correlation with the difference of their parental lines. The oil content of three type of induced kernels (haploid, crossed and hetero-fertilized kernels) were further compared, the results demonstrated that the oil content of hetero-fertilized kernels was significantly greater than that of crossed kernels and haploid kernels, indicating the oil content has obvious xenia, and the xenia contributes more in kernel oil content than dosage effect in maize.

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### **ZmMADS69 functions as a flowering activator through the ZmRap2.7-ZCN8 regulatory module and contributes to maize flowering time adaptation**

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Flowering time is a major determinant of the local adaptation of plants. Although numerous loci affecting flowering time have been mapped in maize, their underlying molecular mechanisms and roles in adaptation remain largely unknown. Here, we report the identification and characterization of MADS-box transcription factor *ZmMADS69* that functions as a flowering activator through the *ZmRap2.7-ZCN8* regulatory module and contributes to adaptation. We show that *ZmMADS69* underlies a quantitative trait locus controlling the difference in flowering time between maize and its wild ancestor, teosinte. Maize *ZmMADS69* allele is expressed at a higher level at floral transition and confers earlier flowering than the teosinte allele under long days and short days. Overexpression of *ZmMADS69* causes early flowering, while a transposon insertion mutant of *ZmMADS69* exhibits delayed flowering. *ZmMADS69* shows pleiotropic effects for multiple traits of agronomic importance. *ZmMADS69* functions upstream of the flowering repressor *ZmRap2.7* to downregulate its expression, thereby relieving the repression of the florigen gene *ZCN8* and causing early flowering. Population genetic analyses showed that *ZmMADS69* was a target of selection and may have played an important role as maize spread from the tropics to temperate zones. Our findings provide important insights into the regulation and adaptation of flowering time.

Gene / Gene Models described: *ZmMADS69*; GRMZM2G171650

Funding acknowledgement: National Natural Science Foundation of China (NSFC), National Key Research and Development Program of China, Recruitment Program of Global Experts, Fundamental Research Funds for the Central Universities

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## **A sequence-indexed reverse genetics resource for maize: a set of lines with single *Ds-GFP* insertions spread throughout the genome**

(submitted by Charles Du <[duc@montclair.edu](mailto:duc@montclair.edu)>)

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The availability of a mutant line in which a single gene has been disrupted gives biologists a powerful tool in understanding that gene's action. Our NSF-PGRP-funded project is generating and sequence-indexing a collection of *Ds* transposon insertions in transgenic maize by taking advantage of next-generation sequencing (NGS) technologies. Almost 200 lines carrying a transpositionally active, marked *Ds-GFP* (*Ds\**) element have been generated and the location of 86 launching platforms has been mapped to all 20 chromosome arms of the maize genome. Over 600 *Ds* transpositions were produced from each of several platforms scattered across the genome. A set of 16,000 transposed *Ds\** (*trDs\**) elements was selected for mapping to the reference genome using a strategy that takes advantage of unique sequences in the *Ds\** element to specifically amplify maize sequences adjacent to the *trDs\** element (*dsg* sites), thereby avoiding amplification of junctions from endogenous elements. The amplified *Ds\** junctions were sequenced in 3-D pools or "cubes" of 960 individuals (arranged in ten 96-well plates, i.e., 96 samples per plate pool x 120 samples per row pool x 80 samples per column pool) and mapped to the B73 reference genome using software, InsertionMapper, developed specifically for the project. Of the 16000 *dsg* sequences, 14184 (~90%) have been unequivocally indexed to a specific cell in the 3-D pool and mapped to the maize genome, illustrating the efficiency of NGS and 3-D pooling for sequence-indexing a large collection of *Ds\** insertions. All the information on sequence-indexed *dsg* sites, including their matching transposant lines, are shared via a web browser hosted at Montclair State University (<http://acdsinsertions.org>). We have set up a MaizeGDB-compatible relational database for the sequence-indexed transposant lines by using the freely available MySQL software. The user interface includes web searching forms written in Java and BLAST search tools.

Funding acknowledgement: National Science Foundation (NSF)

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## **An examination of the effects of epigenetic modifications and active transposable elements on meiotic recombination in maize**

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Meiotic recombination generates genetic diversity and ensures the accurate segregation of homologous chromosomes. Meiotic crossovers are not uniformly distributed along chromosomes. There are hotspots in the hypomethylated chromosomal arms and suppression in heterochromatic regions. The causes of this variation is not well understood, but epigenetic modifications such as DNA methylation and chromatin states are thought to be important factors. In maize, we have examined the effects on meiotic recombination of mutations in a component of the RNA directed DNA methylation pathway, *Mop1* (*Mediator of paramutation1*), a putative RNA-dependent RNA polymerase. The MOPI protein is required to produce an abundant class of small RNAs that are required to maintain methylation of transposable elements (TEs) in regions immediately up and downstream of genes. We found that meiotic recombination was uniformly decreased in the heterochromatic regions of all the 10 maize chromosomes but was increased in most of the examined euchromatic regions. Interestingly, we also found DNA methylation may affect sex-specific differences in the frequency of meiotic recombination, suggesting that these differences may have an epigenetic component. In addition, meiotic recombination is initiated by the formation of DNA double strand breaks (DSBs) and is completed by the repair of these breaks during meiosis. TEs can be a source of DSBs as well. We have developed two F2 populations derived from parents with a large numbers of active or silenced *Mutator* (*Mu*) transposons to test that widely scattered active *Mu* transposons can stimulate overall increases in recombination by comparing recombination with and without transposons and with and without *Mu* activity in maize.

Funding acknowledgement: National Science Foundation (NSF)

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## Analysis of polymorphic TE insertions in maize reveals family specific influences on chromatin insertion site preference and spreading of DNA methylation

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Maize is an important crop species with a complex genome organization. A majority (>64%) of the maize genome is comprised of transposable elements (TEs), most of which are highly methylated. These TEs are interspersed with unmethylated genic regions. We are interested in understanding the interactions between genes and transposons and the role DNA methylation plays in these interactions. These interactions could include how chromatin influences the insertion site preference of TEs and how TE insertions could influence the spreading of DNA methylation. The analysis of CG and CHG methylation levels flanking TE families reveals three patterns characterized by high, moderate, or decreasing methylation. The proportion of TIR families with the decreasing methylation pattern is significantly higher than that of LTR families. The TE families that display these three patterns also show differences for other chromatin modifications within the TE itself. The differences in these patterns could be due to variable insertion site preference or variability of the influence TEs have on presence of flanking DNA methylation. A set of >60,000 polymorphic TE insertions among B73, Oh43, W22 and PH207 were utilized to study chromatin at the haplotypes with and without the TE. The analysis of DNA methylation at haplotypes without the TE reveals that some families have a strong preference to land in lowly methylated regions while others appear to land more prominently in highly methylated regions. For insertions that have occurred at unmethylated regions we can assess haplotypes containing the TE to determine whether DNA methylation spreads to previously unmethylated flanking regions. Many of the TE insertions are associated with DNA methylation spreading with a higher frequency of spreading observed for LTRs compared to TIRs. These analyses reveal a complex interplay between pre-existing chromatin state and the influence a TE exerts on nearby chromatin.

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## Characterization of b1 tandem repeat chromatin proteome in enhancement, silencing, and paramutation

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The control of gene expression by cis-regulatory elements is a shared feature across the kingdoms of life. The maize *booster1* gene (*b1*) is a mechanistic model for control of gene expression from a distal cis-regulatory element. Two epialleles of *b1* produce distinct tissue-specific pigmentation phenotypes and have been well studied over the past 50 years. The *B-Intense* (*B-I*) allele is highly transcribed, while the *B'* is lowly transcribed. When combined in the same nucleus, *B'* paramutates *B-I*, converting the *B-I* allele to *B'*. Expression and paramutation of *b1* is influenced by a hepta-tandem repeat sequence (b1TR) located ~100kb upstream of the *b1* TSS. Differences in DNA-methylation and chromatin structure have been observed at the b1TR in *B'* and *B-I* plants. It is likely that enhancement and silencing of *b1* each rely on distinct trans-factors that may interact with b1TR and modify local chromatin structure. To identify b1TR-interacting proteins, we have developed a discovery-based proteomics approach that utilizes a transgenic copy of b1TR adjacent to a GAL4-upstream activation sequence to capture b1TR chromatin. Chromatin isolated from transgenic *B'* and *B-I* plants will be used as input for chromatin-immunoprecipitation coupled with mass-spectrometry. This approach will allow us to identify b1TR-interacting proteins associated with enhancement, silencing, and paramutation. Preliminary results have identified several proteins that may play a role in *b1* regulation by interacting with b1TR.

Gene / Gene Models described: *b1*; GRMZM2G172795

Funding acknowledgement: National Science Foundation (NSF)

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## Dissecting the maintenance of MuDR transposon silencing in maize

(submitted by Wei Guo <[guo342@purdue.edu](mailto:guo342@purdue.edu)>)

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Transposons make up a substantial portion of most plant genomes. Due to their mutagenic potential, most of them are silenced. Although we know a lot about the means by which transposable elements are silenced, little is known about how their silencing status is maintained. Once reactivated, what accounts for a somatically or a transgenerationally transmitted reactivation state? Here, we use a minimal Mutator line that includes a naturally occurring variant of the MuDR transposon that can heritably trigger epigenetic silencing of that transposon. MuDR carries two genes *mudrA* and *mudrB*. We demonstrated that Mediator of Paramutation1 (MOP1), a putative RNA-dependent RNA polymerase-encoding gene, is required for the maintenance of *mudrA* silencing. However, silenced *mudrA* is only progressively reactivated after multiple generations in a *mop1* mutant background. In contrast, *mudrB* never becomes reactivated. We find that all DNA methylation is lost at *mudrA* in *mop1* mutants in the first generation. Despite this, *mudrA* remains transcriptionally silenced in this generation. Remarkably, we find that this reactivation can be dramatically accelerated in seedlings carrying a silenced MuDR element in a *mop1* mutant background after a brief exposure to high temperature. In contrast to previous observations, in heat stressed plants, both *mudrA* and *mudrB* are reactivated. This active state is maintained throughout the life of the plant after the initial trigger has disappeared. Remarkably, this activity is transmitted to the next generation and is not associated with DNA methylation. This is intriguing because *mudrA* and *mudrB*, which are associated with two mutually exclusive histone marks, are both reactivated upon heat exposure, suggesting that heat stress might integrate two distinct epigenetic pathways to wake up a silenced transposon. Taken together, this project will give us an opportunity to better understand the maintenance of transposon silencing, and the relationship between epigenetic silencing and stress response.

Gene / Gene Models described: *mop1*, *hsp90*; GRMZM2G042443, GRMZM5G833699

Funding acknowledgement: National Science Foundation (NSF)

P368

## DNA methylation footprints during maize domestication and improvement

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DNA methylation is a ubiquitous feature of plant genomes, which plays a critical role in controlling gene expression, plant development, and likely phenotypic variations. However, the evolutionary forces in shaping the variations and landscapes of DNA methylation are largely unknown. To examine the epigenetic variations during maize domestication and improvement, we obtained whole genome bisulfite sequencing (WGBS) data on 53 samples, including the wild ancestor of maize --- teosinte (*Zea mays ssp. Parviglumis*; N=20), Mexican landraces (N=17), and modern maize inbred lines (N=16). Through population-wise comparisons, we identified about 6,000 differentially methylated regions (DMRs), including 2,061 DMRs in CG context, 2,896 in CHG context and 1,072 in CHH context. These DMRs overlap with 2,194 genes, 244 (11.12%) of which are located within selective sweeps. Our preliminary analysis showed that hypermethylated DMRs in maize have a higher genetic load compared to randomly selected genomic regions with the similar features, while hypomethylated DMRs in maize have lower genetic load. Our comparative epigenetic analysis would expand the current knowledge about crop evolution and might provide new opportunities for future maize improvement.

Funding acknowledgement: United States Department of Agriculture (USDA)



P369 

## **Dynamic response to extended drought and recovery involve epigenetic control in stress adaptation and flowering regulation, providing insights into epigenetic memory in maize**

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During their lifespan, plants are exposed to a multitude of stressful factors that affects their development and reproductive fitness. When subjected to stressful conditions, plants rapidly respond and adapt through a sophisticated variety of physiological, biochemical, transcriptional, and epigenetic mechanisms.

Dynamic changes in chromatin structure and concomitant transcriptional variations play an important role not only in stress response, but are also involved in epigenetic memory mechanisms. Histone marks and gene expression patterns could be indeed stably maintained once the triggering stimulus has been removed. There is good evidence that chromatin may play a pivotal role in somatic memory phenomena and although many progresses have been made in understanding chromatin modifications implicated in plant response to environmental triggering conditions, we are still far from connecting molecular genetics and developmental data around environment and chromatin.

Through the integration of RNA-Seq and ChIP-Seq data from maize plants subjected to a mild and prolonged drought stress and after the complete recovery from the stress, we identified several stress-responsive genes in which stress-induced transcriptional and histone marks variations persist after the stress removal and the recovery stage. The stress-induced stable chromatin environments and expression of these genes could represent a coordinated strategy for plants to cope with drought stress and rapidly adapt to recurring stresses.

Drought stress caused transcriptional and chromatin changes also at many genes involved in flowering regulation and inflorescences patterning. Particularly interesting is the stress induced delay of chromatin marks level variation that are normally associated to the increase in expression levels of two flowering associated MADS-box coding genes: both chromatin marks and the associated mRNA levels persist after the stress removal, underlining stress memory and resulting in the stress induced alteration of flowering time and inflorescence development.

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## **Dynamics of inheritance for small RNAs in maize breeding populations and their association with quantitative trait variation**

(submitted by Bosen Zhang <[bszhang@illinois.edu](mailto:bszhang@illinois.edu)>)

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Small RNAs (sRNAs) are ubiquitous components for regulating plant development and maintaining genome integrity, whose functions can vary depending on their length and sequence. To understand inheritance patterns for maize small RNAs in typical breeding populations, we performed deep sequencing of small RNAs from developing ear tissues in three families derived from crosses between the maize inbred lines B73, Mo17, and PH207. We observed dramatic variation for both 21-22nt and 23-24nt sRNAs among these parents, their hybrids, and progeny individuals sampled at the F2, F4 and F6 generations, demonstrating the variable behavior of epigenetic programs during maize inbreeding.

We find that many of the prominent properties observed in small RNA profiles display high heritability, including relative sRNA abundance among transposable element families, accumulation of 23-24nt sRNAs immediately upstream of transcription start sites, and the “sequence depth” of 21-22nt sRNAs. However, we also observed different patterns of TE-derived sRNAs among each of the three pedigrees, where the results are consistent with a ‘wash-in’ or ‘wash-out’ hypothesis for the evolutionary dynamics of epigenetic components. To gain a better understanding of how small RNA variation might contribute to phenotypes, we employed co-expression network analysis of small RNAs, mRNAs, and three years of trait data collected from the individuals in the three pedigrees. This analysis identified modules of TE-sRNAs that are strongly associated with growth phenotypes, including the RLG00010 transposon family that has been reported to be a candidate enhancer element. Finally, we extended our approach of sRNA co-expression and tree correlation analyses to data from hybrids derived from crosses of recent ex-PVP hybrids, which identifies sRNA properties that are associated with yield related traits. Collectively, our results indicate that sRNAs display high heritability, yet also dynamic variation that can be associated with important phenotypes in populations representative of modern maize breeding programs.

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



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## Epigenetic regulation of ABA-induced transcriptional responses in maize

(submitted by Stefania Vendramin <[svendramin@bio.fsu.edu](mailto:svendramin@bio.fsu.edu)>)

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Plants are often subjected to extreme environmental conditions and must adapt rapidly. The phytohormone abscisic acid (ABA) accumulates under abiotic stress conditions, signaling transcriptional changes that trigger physiological responses. Changes in epigenetic modifications are required to facilitate transcriptional responses, particularly at genes exhibiting temporal, tissue-specific, and environmentally-induced expression. In maize, MEDIATOR OF PARAMUTATION 1 (MOP1) is required for progression of an RNA-dependent epigenetic pathway that regulates transcriptional silencing of loci across the genome. To identify genome-wide ABA-induced, MOP1-dependent, and independent transcriptional responses; gene expression profiles were compared between *mop1-1* mutant and *Mop1* seedlings subjected to exogenous ABA. 3,242 differentially expressed genes were identified in four pairwise comparisons of genotype and ABA-treatment, and almost half of them are unique to one group. The majority of the gene expression changes were observed in ABA-treated *mop1-1* mutants, including many transcription factors, suggesting a combinatorial effect caused by ABA-mediated responses and the loss of MOP1. To correlate these changes, a gene regulatory network was used to construct an ABA-response transcription network, and the similarity between this network and a hierarchical model proposed in other plants supports the idea that ABA and MOP1 act to induce extensive primary and secondary transcriptional responses. Correlations between differential expression and potential regulatory RNAs were identified, and the results suggest that RdDM might actively regulate many genes identified in this study. Coordination between ABA- and MOP1-mediated regulatory pathways reinforces the idea that epigenetic regulation is crucial to plant response and adaptation to abiotic stress. These results shed some insight onto the nature of combinatorial, and potentially synergistic, regulation of gene expression in maize.

Funding acknowledgement: National Science Foundation (NSF), Start-up funds from University of Washington, Bothell School of STEM

P372

## Genome-wide hypermethylation of TEs and centromeres following low temperature exposure in maize

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Transposable elements (TEs) are major players in shaping genome structure. TE sequences are transcriptionally silenced by epigenomic modifications to limit the mutagenic potential of their transpositional activity. In particular, several DNA methylation pathways are responsible for TE silencing in the various chromosomal locations where TE reside. While DNA methylation is known to be modified by abiotic constraints, the extent to which it can be remodeled remains to be fully elucidated.

We show that low temperature triggers genome-wide hypermethylation in maize, mainly at transposable elements and centromeres. This hypermethylation is mediated by the parallel activation of multiple methylation pathways across chromosomes, to actively hypermethylate TEs in the various chromatin locations where they reside. This likely reflects the importance of taming transposable elements following an abiotic stress in maize.

Funding acknowledgement: Agence Nationale pour la Recherche (ANR)

**P373**

### **Heterochromatic 24nt siRNAs without RdDM in rice and maize**

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24nt siRNAs are a hallmark of RNA-directed DNA methylation (RdDM) and are typically concentrated at boundaries of heterochromatin, often at the ends of transposons near genes. We found that chromomethylase or DDM-type nucleosome remodeler mutants that disrupt heterochromatin lose 24nt siRNAs from heterochromatin boundaries and gain them in other normally heterochromatic areas. Independently, in our work on small RNAs in wild-type rice gametes, we found similar redistributions of 24nt RNAs as in maize heterochromatin mutants. Neither in maize mutants (in embryo or endosperm) nor in rice gametes do the siRNAs direct DNA methylation, as indicated by lack of methylation in the CHH context. These findings indicate that 24nt siRNAs, likely including ones transcribed by pol IV, do not necessarily recruit DNA methyltransferases, and they raise the possibility of other gene regulatory activities.

Gene / Gene Models described: GRMZM2G025592, GRMZM2G005310, GRMZM2G177165, GRMZM2G071025

Funding acknowledgement: National Science Foundation (NSF)

**P374**

### **Identification and characterization of regulatory sequences in *Zea mays***

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While most cells in multicellular organisms carry the same genetic information, in each cell-type only a subset of genes is being transcribed. Such differentiation in gene expression depends, for a large part, on the activation and repression of cis-regulatory sequences, including transcriptional enhancers. Transcriptional enhancers can be located tens of kilobases from their target genes, but display characteristic chromatin and DNA features, allowing their identification by epigenomic profiling. We have shown that integration of genome-wide DNA methylation, histone acetylation and chromatin accessibility data sets can be used to predict tissue-specific distal enhancer candidates in *Zea mays*. About 1,500 putative regulatory sequences have been identified in young seedling and husk tissue. These include known and experimentally validated enhancers, such as the b1 and tb1 enhancers. Enhancer candidates are characterized by low DNA-methylation, increased chromatin accessibility, and enrichment of H3K9ac. Unlike in animal systems, most enhancer candidates have an asymmetric distribution of H3K9ac and produce enhancer RNAs in a unidirectional manner. Furthermore, maize enhancer candidates overlap with unmethylated regions (UMRs) rather than low-methylated regions (LMRs), as observed for mammalian enhancers. UMRs are generally stable, consistent with maize enhancers being stably unmethylated. Currently, the function of Vgt1, a predicted regulatory element located about 70 kb upstream of the floral repressor gene *ZmRap2.7*, is being studied in more detail. Silencing of Vgt1 by DNA methylation results in earlier flowering and is associated with upregulation of *ZMM4* expression, consistent with Vgt1 acting as an enhancer of *ZmRap2.7*. However, RT-qPCR analyses of several different tissues did not yet reveal a change in *ZmRap2.7* expression levels, raising the question if Vgt1 affects flowering time through other genes than *ZmRap2.7*.

Gene / Gene Models described: *ZmRap2.7*; Zm00001d010987

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## Maize centromeres expand in the larger genome background of Oaxaca and *Zea luxurians*

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Little is known about how centromere size and location are determined. The previous study has revealed that centromere size among grass species is highly correlated with total genome size, and maize centromeres expand when transferred into a larger genome background of oat. However, whether centromeres will expand if we intentionally enlarge the genome size of a specific species is still unknown. In this study, we test the hypothesis that centromere size positively correlates with genome size in *Zea* species by introducing the chromosomes of B73 into Oaxaca and *Zea luxurians*, both Oaxaca and *Zea luxurians* have a larger genome than B73. To increase the genome size while keeping centromere homozygotes from B73, we have crossed B73 with Oaxaca and *Zea luxurians* several times. The genome sizes of these different lines have been estimated by the amount of DNA in cell nuclei from flow cytometry. The results showed that the genome size is bigger in the hybrids of B73 X Oaxaca and B73 X *Zea luxurians* than in B73. The CENH3-ChIP experiment results suggested that four centromeres (centromere 2, 3, 8, 9) are expanded in the BC1F2 hybrids of B73 and Oaxaca, F2 of B73 and *Zea luxurians*. To find the underlying reason for the centromere expansion, we conducted qPCR to detect the transcriptional expression level of centromeric genes in these hybrids, however, their transcriptional expression levels are not affected. These data provide new insights into the relationship between centromere size and genome size in *Zea* species.

Funding acknowledgement: National Science Foundation (NSF)

P376

## MOP1-mediated transcriptional regulation of developmental genes under abiotic stress

(submitted by Thelma Madzima <[madzima@uw.edu](mailto:madzima@uw.edu)>)

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MEDIATOR OF PARAMUTATION 1 (MOP1) is required for progression of an RNA-dependent epigenetic pathway that regulates transcriptional gene silencing at genomic loci in maize. *mop1-1* mutants display pleiotropic transcriptional and developmental phenotypes, indicating that MOP1 is required for normal plant growth and development. The developmental defects observed by loss of MOP1-mediated epigenetic regulation appear to be a result of mis-expression of key regulators of tissue-specific gene expression, and many of the observed physiological phenotypes affect both tassel and ears inflorescences. Consistently, several genes that are key regulators of inflorescence development in maize are differentially expressed in *mop1-1* immature ears, including the floral homeotic genes *ramosa 1* (*ra1*) and *barren stalk fastigiate 1* (*baf1*). *mop1* mutant developmental phenotypes are not predictable, vary in intensity and appear to be influenced, in-part, by abiotic environmental conditions. To investigate the interactions between MOP1-mediated regulation and abiotic stress responses, we subjected *mop1-1* and *Mop1* homozygous seedlings to exogenous treatment with the plant 'stress' hormone abscisic acid (ABA), followed by RNA-sequencing (Vendramin et al.). ~3,000 differentially expressed genes (DEGs) were identified in pairwise comparisons of genotype and ABA-treatment. Genes downregulated in the *mop1-1* + ABA were enriched with unique GO terms associated with growth, development, and reproduction. The enrichment of development-related genes that are differentially expressed between mutants and wildtype under ABA treatment likely correspond to the pleiotropic developmental defective phenotypes observed in *mop1-1* and the thousands of mis-regulated genes as a consequence to the loss of MOP1 activity. However, these regulatory networks are complex, and are subject to ongoing analysis, for which progress will be reported.

Vendramin S, Huang J, Madzima TF, McGinnis KM. Epigenetic Regulation of ABA-Induced Transcriptional Responses in Maize. (submitted)

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## The divergence of NAM founders revealed by syntenic LTR retrotransposons

(submitted by Shujun Ou <[oushujun@iastate.edu](mailto:oushujun@iastate.edu)>)

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Maize (*Zea mays* L.) exhibits extensive molecular variation that is associated with its diverse phenotypes. To understand these associations and facilitate predictive crop breeding, a total of 25 diverse inbred lines (e.g., tropical, temperate, popcorn, sweet corn) were crossed with the B73 inbred line to develop families of recombinant inbred lines (RILs). Collectively these RILs are known as the maize Nested Association Mapping (NAM) population, which has been used extensively to map the genetic basis of complex traits. Due to advances in sequencing and assembly techniques, we were able to generate chromosome-scale genomes of all 26 NAM founder lines. These assemblies provide an opportunity to characterize the evolutionary history of modern breeding material, which is broadly sampled by the NAM founders. In this study, we utilized long terminal-repeat retrotransposons (LTR-RTs) found in syntenic genomic regions to study the divergence of the 26 NAM founder genomes. LTR-RTs are interspersed repetitive elements dominating the maize genome. The frequent insertional activity of LTR-RTs creates thousands of genetic footprints that record the genomic history of maize, such as chromosomal divergence, relocation, and introgression. Further, random mutations that have occurred in the direct repeat of LTR-RTs can serve as ideal molecular clocks to estimate the timing of these events. We reveal that standing LTR diversity from teosinte is likely still segregating in maize, with divergence time between temperate maize lines predating the domestication of maize. The high contiguity of the NAM founder assemblies allowed for complete analysis of the repeat space, an impossibility in previous resequencing studies and has expanded our understanding of the evolution of the repetitive fraction of the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

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## The landscape of centromeres and pericentromeres of *Zea mays*

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Maize centromeres are characterized by frequent and recent neocentromere formation near the ancestral centromeres. Because maize centromeres are highly enriched for the tandem repeat CentC and a group of evolutionarily related centromeric retrotransposons (CR), automated genome assembly leaves gaps, even in the excellent B73 RefGen\_v4 reference genome constructed from long-read data. Manual editing of >136 Mb spanning the ten centromeres of maize inbred B73 resulted in the closure of 127 sequence gaps and the addition of >8.4 Mb of previously unanchored sequence (unitigs and reads) containing 24 genes, 2 Mb of CR repeat and 887 kb of CentC. The functional centromeres of five maize chromosomes were closed completely, including a 7 Mb region spanning CEN2. The improved sequence data from CEN1, CEN4 and CEN6 was used to derive a nucleotide substitution rate estimate of 7.3E-08 substitutions per site and year for maize centromeres. A total of 1,742 CR elements were extracted from the B73 v4CEN assembly and analyzed. Post-domestication CR2 insertions into all active centromere regions are six times more frequent than CR1 insertions. SNP analysis of CR2 elements over time revealed a disproportionate expansion of a single haplotype in the past 100,000 years. It is unclear if this is due to early selection of a single centromere, exceptionally high expression of this element, improved transposition frequency, or genetic drift. Multiple different recombinant CR2 elements with target site duplications suggest the occurrence of recombination during transposition, which complicates phylogenetic analyses. Given the low genetic diversity in many centromeres of domesticated maize, these assemblies of the B73 functional centromeres should assist in assembling orthologous regions of other maize inbreds.

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

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## The roles of RNA polymerase IV and the environment in effecting heritable regulatory changes

(submitted by Benjamin Oakes <[oakes.105@osu.edu](mailto:oakes.105@osu.edu)>)

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Paramutation is a poorly understood behavior in which one parental allele at a given locus induces a meiotically heritable change at the other.<sup>1</sup> Such behaviors occur at several maize loci encoding transcriptional activators of flavonoid biosynthesis pathways including *red1* (*r1*), *booster1* (*b1*), *purple plant1* (*pl1*), and *pericarp color1* (*p1*).<sup>2</sup> Remarkably, Bernard C. Mikula showed that the extent of heritable changes brought about by paramutations occurring at *r1* is influenced by the environment in early development.<sup>3</sup> Because RNA polymerase IV (RNAP IV) controls the heritable regulatory status of the *P11-Rhoades* (*P11-Rh*) allele<sup>4,5,6</sup> – an allele subject to paramutation – we hypothesize that RNAP IV, and potentially small RNA pathways in general, mediate trans-generational changes influenced by the environment. To test this idea, we applied mutant analysis and similar experimental strategies used by Mikula<sup>3</sup> to study environmental effects on *pl1* paramutation and genome-wide heritable changes. B73 BC<sub>4</sub>F<sub>2</sub> seedlings segregating for a mutation (*rmr6-1*) in the gene encoding the RNAP IV largest subunit were grown in a growth chamber during the first two or three weeks of development at varying temperatures and photoperiods. Plants were grown at either 32°C or 22°C and treated with either a 12-hour light/dark cycle (LD) or continuous light (LL)<sup>3</sup> and then transplanted to the field. Sibling *rmr6-1* and *Rmr6-B73* homozygotes identified using a CAPS marker were then backcrossed to B73. Seedling RNA profiles of BC<sub>s</sub> progeny from both non-mutant and *rmr6-1* mutant fathers that were treated with various conditions during early development (32°C LL, 32°C LD, 22°C LL, and 22°C LD) will be compared to identify environment- and RNAP IV-mediated effects on the genome-wide inheritance of regulatory information. These results will address a potential molecular mechanism effecting trans-generational variation in response to environmental change.

1. Brink *Genetics* (1956). | 2. Hollick *Nat. Rev. Genet.* (2017). | 3. Mikula *Genetics* (1995). | 4. Hollick *et al. Genetics* (2005). | 5. Erhard *et al. Science* (2009). | 6. Erhard *et al. Plant Cell* (2013).

Funding acknowledgement: National Science Foundation (NSF)

P380

## TIR-Learner, a new ensemble method for TIR Transposable Element annotation, provides evidence for the impact of TIR TEs on Maize Genome Structure and Evolution

(submitted by Weijia Su <[weijia@iastate.edu](mailto:weijia@iastate.edu)>)

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Transposable elements (TEs) make up a large proportion of plant genomes. There are two major types of TE: Class I TEs transpose via RNA intermediates and are often called RNA transposons or retrotransposons, while Class II TEs do not use RNA transposition intermediates, and are therefore termed DNA transposons. Class I TEs are the dominant TE type in maize genomes. With the explosion of sequence data, many useful tools been developed for class I TE identification and classification, thus the class I TEs are well annotated in many assembled maize genomes. Studies have shown that Class II elements also play an important role in genome evolution, including generating structural variations and regulating gene expression. However, due to the lack of effective tools, the current annotation of Class II TEs is still unsatisfactory, resulting in the underestimation of the true proportion of Class II TEs in the genome and inaccurate TE signature identification. In this poster, we focus on the major components of Class II TEs: TIR elements, which are flanked by Terminal Inverted Repeats (TIR). We describe TIR-Learner, a new ensemble method of TIR element detection and annotation in maize genomes. This new pipeline combines the advantage of homology-based methods with the power of machine learning, thereby greatly increasing the efficiency and accuracy of TIR elements annotation. This improved annotation shows that the actual proportion of DNA elements in maize is much larger than the estimates of current published annotations. We show that TIR TE superfamilies have distinct distributions across the genome and between inbred lines, indicating their major contribution to maize genome diversity. This research is supported by the USDA National Institute of Food and Agriculture Hatch project number IOW05282, and by State of Iowa funds.

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## Transcriptional regulation of the maize genome

(submitted by Allison McClish <[mcclish.23@osu.edu](mailto:mcclish.23@osu.edu)>)

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In both maize<sup>1</sup> and *Arabidopsis*<sup>2</sup>, Global Run-On Sequencing (GRO-Seq) profiles have defined genomic regions where nascent transcription rates are influenced by RNA polymerase IV (Pol IV). Short-lived Pol IV RNAs are processed into 24 nucleotide (24nt) sizes that, in *Arabidopsis*, facilitate RNA-directed cytosine methylation<sup>3</sup>. Although maize Pol IV affects Pol II transcription globally and across specific genes<sup>1</sup>, it remains unclear whether Pol IV itself or Pol IV-dependent cytosine methylation is responsible<sup>4</sup>. GRO-Seq profile comparisons between *dicer-like3* (no 24nt RNAs) and *rpm1* (no Pol IV) mutants should distinguish these two general mechanisms. Additionally, profiles of specific mutants should identify Pol II transcription specifically affected by either Pol IVa or Pol IVb subtypes<sup>5,6</sup>. Current efforts to optimize reactions needed to create high-quality sequencing libraries will be presented. By hybridizing radiolabeled nascent RNAs to single-gene riboprobes, the ideal concentration of sarkosyl — a reported transcription initiation inhibitor<sup>7</sup> — is being identified<sup>8,9</sup>. Nuclei isolated from various tissues are also being evaluated for *in vitro* transcriptional competency. GRO-Seq profiles generated from alpha-amanitin (Pol II-specific inhibitor) -treated nuclei promise to identify rare Pol IV and/or Pol V transcripts. Ultra-deep nascent transcription profiles will assist future maize genome annotations, including gene model validations, enhancer calls, and defining 3' pretermination and intergenic non-coding transcription.

1 Erhard *et al.* 2015 *Genetics* | 2 McKinlay *et al.* 2018 *RNA Biol* | 3 Matzke and Mosher 2014 *Nat Rev Gen* | 4 Hale *et al.* 2009 *PLoS Gen* | 5 Stonaker *et al.* 2009 *PLoS Gen* | 6 Sidorenko *et al.* 2009 *PLoS Gen* | 7 Gariglio *et al.* 1974 *FEBS Let* | 8 Hawley and Roeder 1985 *J Biol Chem* | 9 Szentirmai and Sawadogo 1994 *N-A-R*

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## Transposable element contributions to maize genome variation

(submitted by Sarah Anderson <[sna@umn.edu](mailto:sna@umn.edu)>)

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Transposable elements (TEs) are ubiquitous components of eukaryotic genomes and can create variation in genomic organization. The majority of maize genomes are composed of TEs, and structural TE annotations are available for four maize genome assemblies (B73, W22, Mo17, and PH207). We developed an approach to define shared and variable TE insertions across genome assemblies, identifying 1.6 Gb of variable TE sequence representing a combination of recent TE movement, deletions, and haplotype diversity. Although recent TE movement only accounts for a portion of the TE variability, we identified 4,737 TEs unique to any one genome with defined insertion sites in all other genomes. Variable insertions were found for all TE superfamilies and are distributed genome-wide, including in regions devoid of SNP markers. These variable TEs have the potential to capture genes, and we found 2,380 genes annotated in the B73 genome that are located within variable TEs leading to differences in gene content among genotypes. The large scope of TE variation across these four temperate maize genomes highlights the importance of TEs in contributing to variation in genome organization and gene content.

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## Transposable elements contribute to adaptive response in selected maize populations

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Genomes are riddled with variation - from single nucleotide changes to the insertion of transposable elements or large scale chromosomal rearrangements. This variation is the raw material on which selection can act, but the relative contribution of these types of variants to adaptation is not clear. Here, we address the contribution of transposable elements (TEs) to adaptation in two mass-selected maize populations by assessing allele frequencies of TE insertions and SNPs both before and after 30 generations of selection. Both populations originated from open pollinated varieties of maize varying for hundreds of thousands of TEs not present in the B73 reference genome. One population, initiated from Krug Yellow Dent, was selected divergently for small and large seeds and previously shown to harbor a large number of SNPs that reached fixation due to selection. This pattern persists in our analysis of TE variation, and we observe TEs disrupting genes of known seed function (e.g. opaque endosperm1). Selection in the Golden Glow population -- bred for increased ear number -- was previously shown to occur via multiple mutations or mutations from standing variation. In this population, TE copy number on average decreases after selection, although insertions that disrupt transcription factors increase in frequency. This provides intriguing support for the importance of past selection in shaping extant diversity: seed size has likely been selected in the past in ancestral populations, but increased ear shoot number has largely been selected against. When existing genetic variation is exhausted, new mutations like those contributed by TEs may hasten adaptive response.

Gene / Gene Models described: ; Zm00001d052110

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## Transposon-induced inversions activate tissue specific gene expression in maize

(submitted by Sharu Paul Sharma <[sharu@iastate.edu](mailto:sharu@iastate.edu)>)

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Structural rearrangements of genome caused by DNA transposons such as *Activator (Ac)* element can have considerable biological impacts such as disrupted gene function, change in gene expression (Zuo et al. 2016), or formation of chimeric genes (Wang et al., 2015). We studied the molecular structures of genomic rearrangements caused by alternative transpositions of *Ac/fAc* elements inserted in or near the *p1* and *p2* genes on chromosome 1. The *p1* gene encodes a Myb-type transcriptional regulator of flavonoid pigmentation in kernel pericarp and cob glumes; *p2* is a linked paralogous gene that is expressed in anther and silk, but not kernel pericarp and cob. We have isolated an allele termed *p1-wwB54* in which *p1* is partially deleted while *p2* is intact; the kernel pericarp is white with infrequent red sectors. From *p1-wwB54*, we identified 19 independent inversions that change the position of a *p1* enhancer and activate expression of *p2* in the kernel pericarp, resulting in red kernel color. Inversion endpoints were isolated by PCR and sequenced. Southern blotting experiments revealed presence of multiple copies of the *p1* enhancer in some inversions. Multiple copies of enhancer also correspond to darker red kernel phenotype compared to alleles with single copy enhancers. Future experiments will test how the *p1* enhancers may interact with the *p2* promoter from very different and often long distances to activate gene expression.

For animations of Alternative Transposition, see; <http://thomaspublic.iastate.edu/transposition.html>

Wang et al., 2015. Alternative transposition generates segmental duplications and new chimeric genes at the maize *p1* locus. *Genetics*, 201: 925–935

Zuo et al., 2016. Genes and small RNA transcripts exhibit dosage-dependent expression pattern in maize copy-number alterations. *Genetics*, 203: 1133–1147

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## Understanding centromeric retrotransposons I – identification of protease cut sites and assembly of virus-like particles

(submitted by Ryan Shontell <[ryankhs@hawaii.edu](mailto:ryankhs@hawaii.edu)>)

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Centromere-specific retrotransposon (CR) elements have the amazing ability to target plant centromeres during integration using an as yet unknown targeting mechanism. Better understanding of CR biology and their potential use in maize transformation begins with identifying the different polyprotein (Pol) components that result from autocatalytic digestion. This digestion is performed by the aspartyl-like protease encoded within Pol whose target sites have not yet been experimentally determined. Using protein folding, modeling, and docking software we predicted putative cleavage sites within the polyprotein. Recombinant CR polyprotein expressed in *E. coli* was used for *in vitro* and *in vivo* protease digestion assays to experimentally validate the predicted sites. To date, we have validated the cleavage site separating the Gag from the rest of the polyprotein using mass spectrometry. Based on these data we produced full-length Gag protein that we used to perform VLP assemblies *in vitro*. The resulting pro-VLPs ranged from 36-44 nm in diameter as determined by transmission electron microscopy. The 2018 University of Hawaii undergraduate iGEM (international Genetically Engineered Machines) team contributed to this research.

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## Variation and inheritance of small RNAs in maize inbreds and their hybrids

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Small RNAs (sRNAs) regulate gene expression, play important roles in epigenetic pathways, and have been hypothesised to contribute to hybrid vigor in plants. Here we provide a deep exploration of sRNA variation and inheritance among a panel of 108 maize samples that include data for five tissues from 8 inbred parents and 12 hybrid genotypes, covering a spectrum of heterotic groups, genetic variation, and levels of heterosis for various traits. There is substantial variation in the profile of sRNAs in terms of sRNA size classes and genomic repetitiveness among the different tissues. However, the overall profile of sRNAs is generally similar between inbreds and hybrids in each tissue. Locus-specific abundances of sRNA profiles exhibit patterns of variation that reflect genetic relationships among samples. There is evidence for differentiation of locus-specific sRNA profiles by both genotype and inbred/hybrid suggesting differential accumulation of sRNAs from specific genomic regions in inbreds relative to hybrids. In contrast to mRNA expression data, we identify much higher levels of non-additivity for sRNA abundances, including some loci with sRNA accumulation only in hybrids or inbreds. These results provide a valuable resource for understanding the potential role of sRNAs in hybrid vigor.

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Zhou, Peng	University of Minnesota
Zhou, Yan	Iowa State University
Zuffo, Leandro	Iowa State University

## **History of the Maize Genetics Conference**

Year	Annual	Location	Dates	Chair
2019	61	St. Louis, Missouri	March 14-17	Michael Muszynski
2018	60	Saint-Malo, France	March 22-25	Alain Charcosset
2017	59	St. Louis, Missouri	March 9-12	Erich Grotewold
2016	58	Jacksonville, Florida	March 17-20	David Braun
2015	57	St. Charles, Illinois	March 12-15	Mark Settles
2014	56	Beijing, China	March 13-16	Ann Stapleton
2013	55	St. Charles, IL	March 14-17	Phil Becraft
2012	54	Portland, OR	March 15-18	John Fowler
2011	53	St. Charles, IL	March 17-20	Erik Vollbrecht
2010	52	Riva del Garda, Italy	March 18-21	Jane Dorweiler
2009	51	St. Charles, IL	March 12-15	Steve Moose
2008	50	Washington, DC	February 27 - March 2	Thomas Brutnell
2007	49	St. Charles, IL	March 22-25	Anne Sylvester
2006	48	Asilomar, Pacific Grove, CA	March 9-12	Jay Hollick
2005	47	Lake Geneva, WI	March 10-13	Martha James
2004	46	Mexico City, Mexico	March 11-14	Mike Scanlon
2003	45	Lake Geneva, WI	March 13-16	David Jackson
2002	44	Kissimmee, FL	March 14-17	Sarah Hake and Sue Wessler
2001	43	Lake Geneva, WI	March 15-18	Torbert Rocheford and Sue Wessler
2000	42	Coeur d'Alene, ID	March 16-19	Rebecca Boston and Sue Wessler
1999	41	Lake Geneva, WI	March 16-19	Julie Vogel and Cliff Weil
1998	40	Lake Geneva, WI	March 19-22	Mike McMullen
1997	39	Clearwater Beach, FL	March 13-16	Paul Sisco
1996	38	St. Charles, IL	March 14-17	Paul Chomet
1995	37	Asilomar, Pacific Grove, CA	March 16-19	Karen Cone
1994	36	St. Charles, IL	March 24-27	Kathy Newton
1993	35	St. Charles, IL	March 18-21	Tim Nelson
1992	34	Asilomar, Pacific Grove, CA	March 19-22	Sarah Hake
1991	33	Lake Delavan, WI	March 21-24	Jim Birchler
1990	32	Lake Delavan, WI	March 8-11	
1989	31	Lake Delavan, WI	March 2-5	
1988	30	Madison, WI	March 25-27	
1987	29	Lake Delavan, WI	March 20-22	
1986	28	Lake Delavan, WI	March 21-23	Curt Hannah
1985	27	Lake Delavan, WI	March 29-31	Hugo Dooner
1984	26	Champaign, IL	March 10-11	Earl Patterson
1983	25	Allerton Park, IL	March 12-13	Earl Patterson
1982	24	Allerton Park, IL	March 13-14	Earl Patterson
1981	23	Allerton Park, IL	March 14-15	Earl Patterson
1980	22	Allerton Park, IL	March 8-9	Earl Patterson
1979	21	Allerton Park, IL	March 10-11	Earl Patterson
1978	20	Allerton Park, IL	March 11-12	Earl Patterson
1977	19	Allerton Park, IL	March 12-13	Earl Patterson
1976	18	Allerton Park, IL	March 13-14	Earl Patterson

Year	Annual	Location	Dates	Chair
1975	17	Allerton Park, IL	March 8-9	Earl Patterson
1974	16	Allerton Park, IL	March 9-10	Earl Patterson
1973	15	Allerton Park, IL	March 10-11	Earl Patterson
1972	14	Allerton Park, IL	March 11-12	Earl Patterson
1971	13	Allerton Park, IL	March 13-14	Earl Patterson
1970	12	Allerton Park, IL	March 14-15	Earl Patterson
1969	11	Allerton Park, IL	March 15-16	Earl Patterson
1968	10	Allerton Park, IL	March 16-17	Earl Patterson
1967	9	Allerton Park, IL	March 11-12	Earl Patterson
1966	8	Allerton Park, IL	March 12-13	Earl Patterson
1965	7	Allerton Park, IL	March 13-14	Earl Patterson
1964	6	Allerton Park, IL	March 14-15	Earl Patterson
1963	5	Allerton Park, IL	March 9-10	Earl Patterson
1962	4	Allerton Park, IL	March 17-18	Earl Patterson
1961	3	Allerton Park, IL	March 18-19	Earl Patterson
1960	2	Allerton Park, IL	March 12-13	Earl Patterson
1959	1	Allerton Park, IL	January 8-9	John Laughnan, Ed Coe, Gerry Neuffer, and Earl Patterson

# Notes

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## **This conference received financial support from:**

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Corteva Agriscience, Agriculture Division of DowDuPont  
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